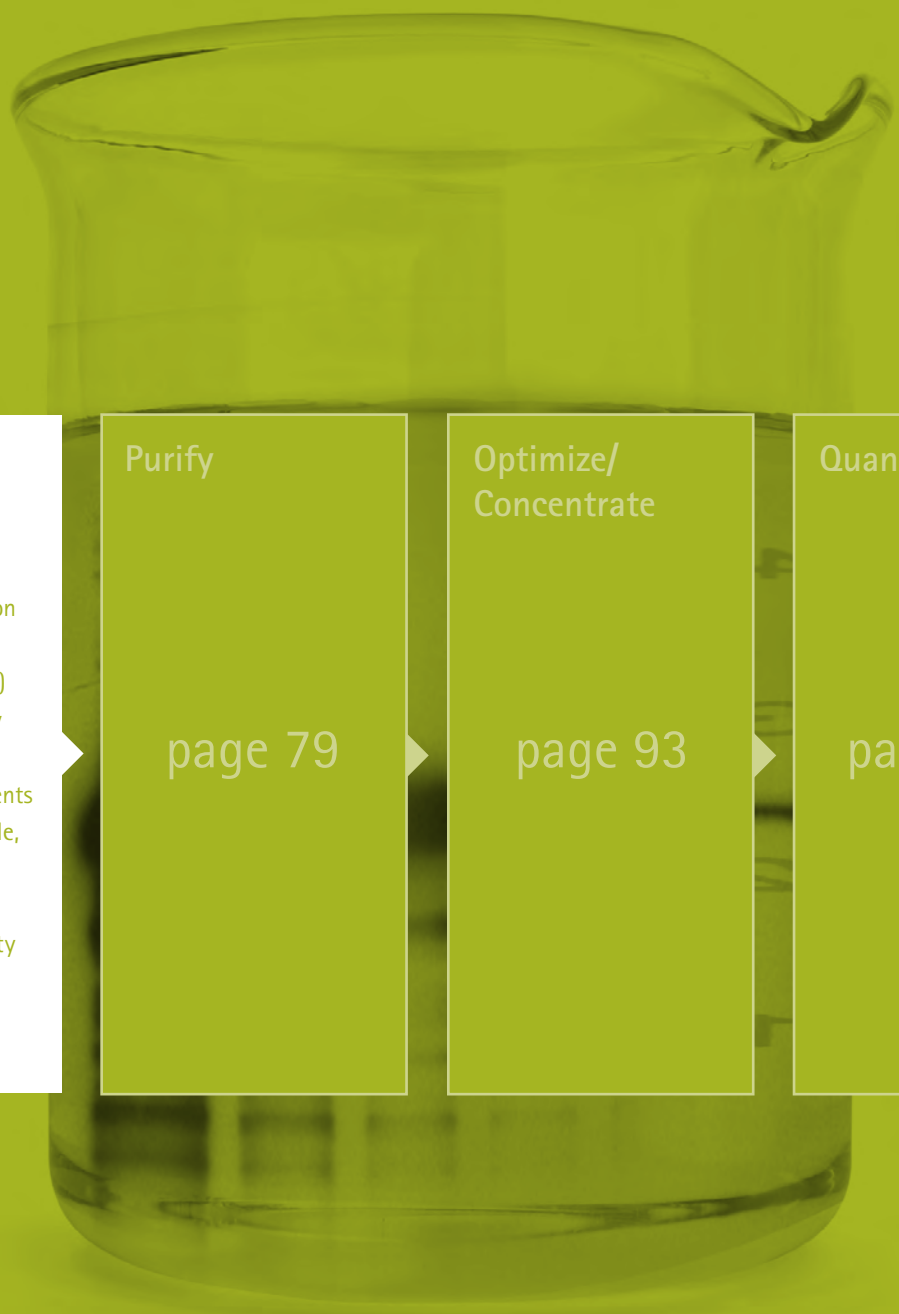
Protein Sample Preparation

High purity and recovery mean better discovery—so start with the best tools for protein purification and preparation. Instead of arduous protocols, focus your efforts on exciting proteome analyses. From protein extraction and protein purification, to protein concentration and desalting, we facilitate every step of your sample preparation workflow with ultrafiltration devices, magnetic beads, extraction kits and more.



Extract

Choosing the right protein extraction protocol can depend on your sample type and your protein analyte(s) of interest. That's why we offer a complete, diverse range of reagents and enzymes for gentle, efficient cell lysis and protein extraction, preserving the integrity and activity of your target protein.

Purify

page 79

Optimize/ Concentrate

page 93

Quantify/Detect

page 111

Protein Extraction Reagents Application Guide

Product	Starting Material: Total Culture	Starting Material: Cell Pellet	Applications: HT Compatible	Applications: Analysis: 1D PAGE	Applications: Analysis: 2D PAGE	Applications: Analysis: IEF	Applications: Analysis: MS	Applications: Analysis: Western Blot	Applications: Analysis: Activity Assay	Applications: Purification	Comments
E. coli											
BugBuster® Protein Extraction Reagent		✔		✔	✔	✔		✔	✔	✔	Efficient protein extraction from *E. coli* under non-denaturing conditions. Extraction enhanced by the addition of rLysozyme™ Solution and Benzonase® Nuclease. Can be used on cell pellets from any size culture.
BugBuster® HT Protein Extraction Reagent		✔	✔	✔	✔	✔		✔	✔	✔	Rapid protein extraction and nucleic acid degradation. Ideal for processing many samples of any volume. Benzonase® Nuclease is premixed in the lysis reagent. Extraction enhanced by the addition of rLysozyme™ Solution.
BugBuster® Master Mix		✔	✔	✔	✔	✔		✔	✔	✔	BugBuster® Master Mix combines BugBuster® Protein Extraction Reagent with Benzonase® Nuclease and rLysozyme™ Solution. Convenient, all-in-one protein extraction reagent efficiently lyses bacteria and digests nucleic acids.
BugBuster® (primary amine-free) Extraction Reagent		✔		✔	✔	✔		✔	✔	✔	Ideal as an extraction method for purifying metal-dependent proteins or proteins to be used for immobilization or crosslinking. Extraction enhanced by the addition of rLysozyme™ Solution and Benzonase® Nuclease.
BugBuster® 10X Protein Extraction Reagent		✔		✔	✔	✔		✔	✔	✔	A concentrated form of BugBuster® Protein Extraction Reagent. Ideal for extraction when a specific buffer is required for protein stability. Extraction enhanced by the addition of rLysozyme™ Solution and Benzonase® Nuclease.
PopCulture® Reagent	✔		✔	✔				✔	✔	✔	Protein extraction from cells directly in the culture medium; no centrifugation required. Designed for small volumes. Extraction enhanced by the addition of rLysozyme™ Solution and Benzonase® Nuclease.
Yeast											
YeastBuster™ Protein Extraction Reagent		✔		✔				✔	✔	✔	Efficient protein extraction from yeast under non-denaturing conditions from any volume of culture. Add 0.5 M THP Solution (included) and Benzonase® Nuclease for enhanced efficiency.
Insect											
CytoBuster™ Protein Extraction Reagent		✔ +		✔	✔ **	✔ **		✔	✔	✔	Gentle lysis of insect cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
Reportasol™ Extraction Buffer		✔ +	✔	✔	✔ **	✔ **		✔	✔ R		Optimized for maximal activity of reporter enzymes (β-gal, firefly, and *Renilla* luciferases). Passive lysis of monolayers.
Insect PopCulture® Reagent	✔		✔	✔				✔	✔	✔	Lysis of insect cells directly in serum-free medium. Ideal for expression screening of many small samples. Compatible with affinity purification.

Key:

1D PAGE = One-dimensional Polyacrylamide Gel Electrophoresis
MS = Mass Spectrometry
R = Reporter Assay
2D PAGE = Two-dimensional Polyacrylamide Gel Electrophoresis

+ = Cell pellet or adherent cells
* = SDS must be removed before IEF
IEF = Isoelectric Focusing G = Gel Shift
** = Salt must be removed before IEF

Protein Extraction Reagents Application Guide

Product	Starting Material: Total Culture	Starting Material: Cell Pellet	Applications: HT Compatible	Applications: Analysis: 1D PAGE	Applications: Analysis: 2D PAGE	Applications: Analysis: IEF	Applications: Analysis: MS	Applications: Analysis: Western Blot	Applications: Analysis: Activity Assay	Applications: Purification	Comments
Mammalian											
CytoBuster™ Protein Extraction Reagent		✔ +		✔	✔ **	✔ **		✔	✔	✔	Gentle lysis of mammalian cells with retention of protein activity for assays and purification. Can use with monolayers or pellets derived from suspension cultures.
Reportasol™ Extraction Buffer		✔ +	✔	✔	✔ **	✔ **		✔	✔		Optimized for maximal activity of reporter enzymes (β-gal, firefly, and *Renilla* luciferases). Passive lysis of adherent cells.
PhosphoSafe™ Extraction Reagent		✔ +	✔	✔	✔ **	✔ **	✔	✔	✔	✔	Ideal for extraction of phosphorylated proteins.
NucBuster™ Protein Extraction Kit		✔		✔ G	✔ **	✔ **		✔	✔	✔	Rapid isolation of nuclear protein fraction from mammalian cells. Ideal for electrophoretic mobility shift assays.
ProteoExtract® Mammalian Complete Proteome Extraction Kit		✔		✔	✔ *	✔ *	✔ *	✔			Total proteome extracted into one fraction.
ProteoExtract® Transmembrane Protein Extraction Kit		✔		✔	✔	✔	✔	✔	✔		Enables mild and efficient extraction of transmembrane proteins such as GPCRs.
ProteoExtract® Subcellular Proteome Extraction Kit		✔		✔	✔ (*)	✔ (*)	✔ (*)	✔	✔		Produces four native protein fractions based on subcellular localization.
ProteoExtract® Native Membrane Proteome Extraction Kit		✔		✔	✔	✔	✔	✔	✔		Produces two native protein fractions, membrane and non-membrane.
Lysis and Extraction Enhancement											
Gram-negative bacteria (*E. coli*)	rLysozyme™ Solution	✔	✔	✔	✔				✔	✔	✔	Cleaves bond in peptidoglycan layer of *E. coli* cell wall. Use alone or combined with BugBuster® or PopCulture® reagents for improved protein extraction. Use with Benzonase® Nuclease to reduce sample viscosity and degrade nucleic acids.
	Lysonase™ Bioprocessing Reagent	✔	✔	✔	✔				✔	✔	✔	Convenient mixture of rLysozyme™ Solution and Benzonase® Nuclease minimizes pipetting steps.
Gram-positive bacteria	Chicken Egg White Lysozyme Solution	✔	✔	✔	✔				✔	✔	✔	Cleaves bond in peptidoglycan layer of bacterial cell wall.
All cells	Benzonase® Nuclease	✔	✔	✔	✔				✔	✔	✔	Degrades all types of nucleic acids for more efficient protein extraction, faster chromotagraphy, and reduced interference in assays.

Key:

1D PAGE = One-dimensional Polyacrylamide Gel Electrophoresis
MS = Mass Spectrometry
R = Reporter Assay
2D PAGE = Two-dimensional Polyacrylamide Gel Electrophoresis

+ = Cell pellet or adherent cells
* = SDS must be removed before IEF
IEF = Isoelectric Focusing G = Gel Shift
** = Salt must be removed before IEF

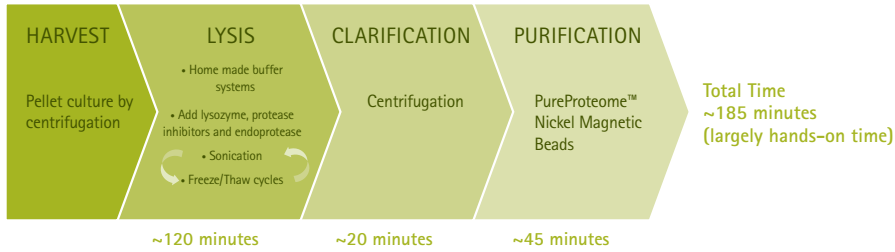
Automated purification of proteins from non-clarified lysate

We developed a one-step lysis protocol using BugBuster® Master Mix to gently disrupt the *E. coli* cell wall while simultaneously reducing lysate viscosity. Subsequently, PureProteome™ Nickel Magnetic Beads could be used to purify recombinant His-tagged proteins without lysate clarification. This "condensed" purification workflow can be automated on systems such as the KingFisher® particle processors, providing fast and reproducible results.

- Reproducible results with minimal hands-on time
- Significant time savings with high yields compared to the traditional workflow
- Magnetic beads eliminate the need to clarify lysates by centrifugation
- Automatable on platforms such as the KingFisher® particle processor

Learn more at:
www.merckmillipore.com/pureproteome

Panel A. Traditional recombinant protein purification workflow with mechanical lysis and clarification.



Panel B. Integrated lysis and purification of non-clarified lysate with magnetic beads.

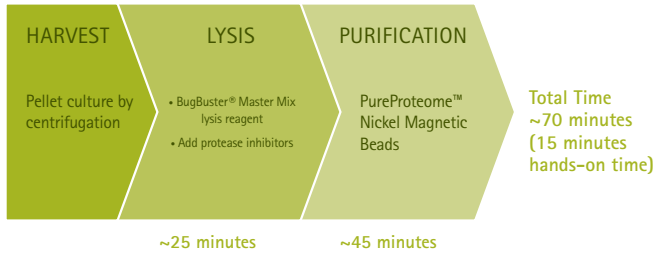


Figure 1. One-step lysate preparation without clarification (Panel B) saves considerable time compared to traditional recombinant protein purification, which requires manual lysis and lysate clarification (Panel A).

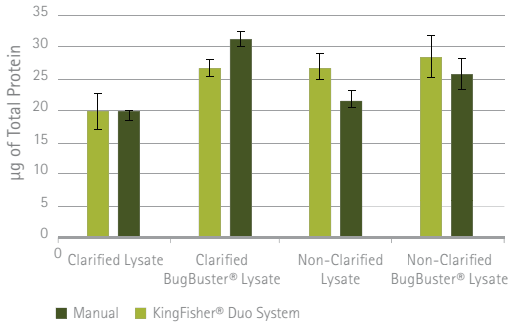


Figure 2. BugBuster® Master Mix generates higher yields of total protein upon purification. Automated processing generated yields comparable to manual processing, and omitting the clarification step provided similar yields.

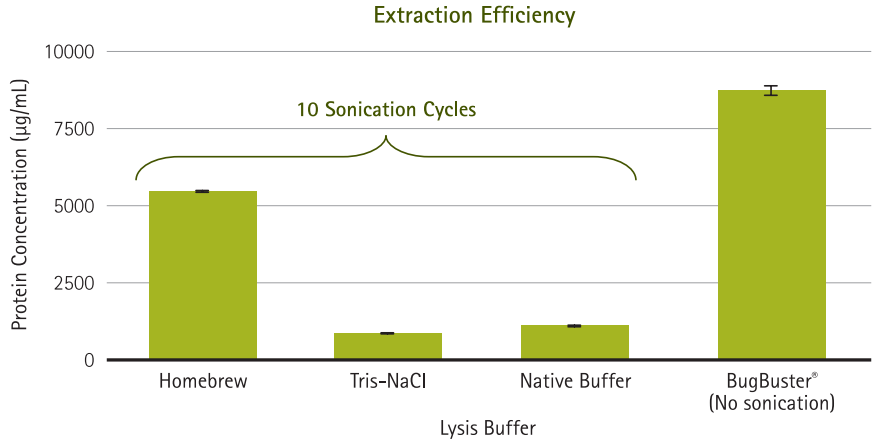
For BugBuster® Master Mix ordering information, please refer to page 71.
For EDTA-free inhibitor cocktail ordering information, please refer to page 76.

BugBuster® Protein Extraction Reagents

Bacterial cell lysis

For gentle, efficient, non-mechanical extraction of soluble proteins from bacterial cells, use BugBuster® Protein Extraction Reagents. This proprietary, innovative, Tris-buffer based mixture of non-ionic and zwitterionic detergents and other ingredients is capable of perforating cell walls without denaturing protein. It provides a simple, rapid, low-cost alternative to mechanical methods, such as French Press or sonication, for releasing expressed target protein for purification or other applications. Simply add the BugBuster® Reagent to your cell pellet and incubate at room temperature for 10 minutes.

Product Performance



Higher extraction efficiency of BugBuster® reagent (with added Benzonase® nuclease and rLysozyme™ solution), even without sonication, compared to three other lysis buffers that were used with 10 cycles of sonication. Cleared cell lysates were spotted on assay cards and quantified using the Direct Detect® spectrometer. Bars represent mean and standard deviation (n=3).

Features & Benefits

- No special sample preparation or instruments are needed
- Standard reagent is supplied as a Tris-buffered "1X" ready-to-use liquid
- Stable at room temperature
- Performance can be enhanced using Benzonase® Nuclease and rLysozyme™ Solution
- Fully compatible with GST•Bind™, Ni-NTA His•Bind®, S•Tag™, Strep•Tactin, and T7•Tag® Resins, or several other chromatography matrices for affinity purification

Applications

Protein Extraction, Protein Sample Preparation, Bacterial Cell Lysis

Ordering Information

Description	Components	Qty/Pk	Catalogue No.
BugBuster® Master Mix	BugBuster® Protein Extraction Reagent, Benzonase® Nuclease, and rLysozyme™ solution in one convenient reagent. For 20 g cell paste	100 mL	71456-3
	BugBuster® Protein Extraction Reagent, Benzonase® Nuclease, and rLysozyme™ solution in one convenient reagent. For 100 g cell paste	500 mL	71456-4
BugBuster® Protein Extraction Reagent	Tris-buffered 1X	100 mL	70584-3
		500 mL	70584-4
BugBuster® Reagent Plus Benzonase® Nuclease	500 mL BugBuster® Reagent and 10 KU Benzonase® Nuclease, Purity >90%. Benzonase® Nuclease is supplied in 50% glycerol containing 50 mM Tris-HCl, 20 mM NaCl, and 2 mM $MgCl_2$, pH 8.0.	1 kit	70750-3
BugBuster® Plus Lysonase™ Kit	100 mL BugBuster® Reagent and 0.2 mL Lysonase™ Bioprocessing Reagent. Use 5 mL BugBuster® Reagent with 10 µL Lysonase™ Reagent. For 20 g cell paste.	1 kit	71370-3
	500 mL BuBuster® Reagent and 1 mL Lysonase™ Bioprocessing Reagent. Use 5 mL BugBuster® Reagent with 10 µL Lysonase™ Reagent. For 100 g cell paste.	1 kit	71370-4
BugBuster® HT Protein Extraction Reagent	BugBuster® Protein Extraction Reagent and Benzonase® Nuclease in one convenient reagent; ideally suited for high-throughput protein purifications.	100 mL	70922-3
		500 mL	70922-4
		1 L	70922-5
BugBuster® (Primary Amine-Free) Extraction Reagent	PIPPS-buffered 1X; will not complex metal ions	100 mL	70923-3
		500 mL	70923-4
BugBuster® 10X Protein Extraction Reagent	10X concentrated formulation of proprietary detergents employed in BugBuster® Reagent without buffer components, allowing user-defined dilution to control pH, reagent concentration, and buffer additives.	10 mL	70921-3
		50 mL	70921-4
		100 mL	70921-5
BugBuster® GST•Bind™ Purification Kit	2 x 100 mL BugBuster® Protein Extraction Reagent; 10,000 U Benzonase® Nuclease, purity >90%; 10 mL GST•Bind™ resin; pkg/4 Chromatography Columns; 2 x 100 mL 10X GST•Bind™/ Wash Buffer; 40 mL 10X Glutathione Reconstitution Buffer; 1 g Glutathione, reduced	1 kit	70794-3

Ordering Information – Continued

Description	Components	Qty/Pk	Catalogue No.
BugBuster® His•Bind® Purification Kit	2 x 100 mL BugBuster® Protein Extraction Reagent; 10,000 U Benzonase® Nuclease, purity >90%; 10 mL His•Bind® resin; 1 His•Bind® Buffer kit; pkg/4 Chromatography Columns	1 kit	70793-3
BugBuster® Ni-NTA His•Bind® Purification Kit	2 x 100 mL BugBuster® Protein Extraction Reagent; 10,000 U Benzonase® Nuclease, purity >90%; 10 mL Ni-NTA His•Bind® resin; pkg/4 Chromatography Columns	1 kit	70751-3
PopCulture® Reagent	Buffered mixture of concentrated detergents formulated to extract proteins from *E. coli* cells directly in their culture medium.	15 mL	71092-3
		75 mL	71092-4
		250 mL	71092-5

Note: BugBuster® Protein Extraction Reagent is compatible with Protease Inhibitors.

For more information visit: www.merckmillipore.com/psp

CytoBuster™ Protein Extraction Reagent

Mammalian and insect cell lysis

Optimized for efficient extraction of soluble, functionally active proteins from mammalian and insect cells, CytoBuster™ Reagent is a gentle, non-ionic formulation that eliminates the need for sonication or freeze/thaw cycling. CytoBuster™ Reagent has been specifically formulated for Western blotting, immunoprecipitation and kinase/phosphatase assays. The reagent is compatible with protease, kinase and phosphatase inhibitors. Related products include the NucBuster™ Kit for nuclear protein extraction in less than 30 minutes, PhosphoSafe™ Reagent for extracting cytosolic proteins while preserving their phosphorylation state and Reportasol™ Buffer for extracting maximal reporter enzyme activity.

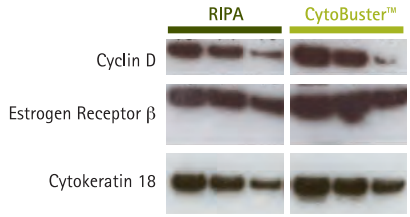
Features & Benefits

- Obtain extracts of native protein from mammalian and insect cells in 5 minutes
- Stable at room temperature
- No need for sonication or freeze/thaw steps

Applications

Protein Extraction, Protein Sample Preparation, Mammalian Cell Lysis, Insect Cell Lysis

Product Performance



More efficient release and/or preservation of breast cancer biomarkers from MCF-7 cells using CytoBuster™ reagent. Immunodetection of cyclin D1 (top panel), estrogen receptor β (middle panel) and cytokeratin 18 (bottom panel) in MCF-7 cell lysates prepared with RIPA buffer or CytoBuster™ reagent.

Ordering Information

Description	Applications	Components	Qty/Pk	Catalogue No.
CytoBuster™ Protein Extraction Reagent*	Mammalian and insect cells	1 bottle	50 mL	71009-3
		5 bottles	100 mL	71009-4
NucBuster™ Protein Extraction Reagent	100 preparations of nuclear extract from 1 x 10^7 to 5 x 10^7 mammalian cells	2 x 7.5 mL NucBuster™ Extraction Reagent 1; 7.5 mL NucBuster™ Extraction Reagent 2; 100 µL 100 mM DTT; 1 set Protease Inhibitor Cocktail Set 1 (lyophilized, makes 100 µL)	1 kit	71183-3
PhosphoSafe™ Extraction Reagent**	Mammalian and insect cells	1 bottle	25 mL	71296-3
		5 bottles	125 mL	71296-4
Reportasol™ Extraction Buffer	Mammalian and insect cells	1 bottle	25 mL	70909-3
		5 bottles	125 mL	70909-4
Insect PopCulture® Reagent	Insect cells	Buffered mixture of concentrated detergents formulated to extract proteins from insect cells directly in their culture medium.	50 mL	71187-3
			250 mL	71187-4

*CytoBuster™ Protein Extraction Reagent is compatible with protease, kinase, and phosphatase inhibitors.

**Phosphosafe™ Reagent includes 4 phosphatase inhibitors: sodium fluoride, sodium vanadate, β-glycerophosphate, and sodium pyrophosphate. Compatible with kinase assays and other applications.

For more information visit: www.merckmillipore.com/psp

YeastBuster™ Protein Extraction Reagent

Yeast cell lysis

For fast, reproducible and gentle extraction of active proteins from yeast and plant cells, use YeastBuster™ Protein Extraction Reagent. This reagent avoids the harsh conditions of vigorous mechanical or chemical treatment that often result in degradation of target proteins. Harvest cells and resuspend in YeastBuster™ protein extraction reagent, incubate for 15 minutes and then remove cell debris by centrifugation. Your extract is now ready to use. The reagent has been tested with *Saccharomyces cerevisiae*, *Pichia pastoris*, *P. stipidis*, and *Schizosaccharomyces pombe* strains and with plant cells.

Features & Benefits

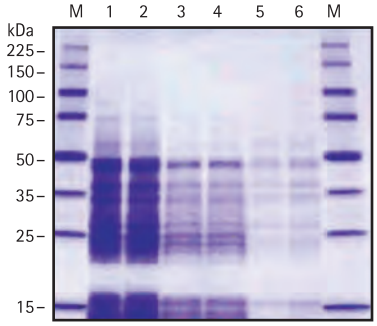
- Eliminates the inconsistencies associated with abrasive grinding, ultrasonication, and pressure disruption of yeast cells
- Higher yield of total and enzymatically active proteins
- Efficient protein extraction from yeast under non-denaturing conditions from any volume of culture
- Add 0.5 M THP Solution (included) and Benzonase® Nuclease for enhanced efficiency
- Fully compatible with Ni-NTA His•Bind® and GST•Bind™ affinity purification method

Applications

Protein Extraction, Protein Sample Preparation, Yeast Cell Lysis, Plant Cell Lysis

Product Performance

A. SDS-PAGE



Lane	Sample
M	Perfect Protein™ Markers, 10-225 kDa
1	5 µL YeastBuster™ Extract
2	5 µL YeastBuster™ Extract
3	5 µL Competitor Reagent Extract
4	5 µL Competitor Reagent Extract
5	5 µL Glass Bead Extract
6	5 µL Glass Bead Extract

B. Protein and Reporter Assays

	YeastBuster™	Competitor	Glass Beads
Protein (mg/mL)	6.1	3.2	0.65
GST (ΔA_{310}/min)	0.071	0.023	0.007
β-gal (ΔA_{310}/min)	0.113	0.003	0.187

YeastBuster™ Protein Extraction Reagent releases more total protein and more recombinant protein activity than another commercial reagent and glass bead extraction. (A) *S. cerevisiae* cells containing a recombinant plasmid expressing a 35.6 kDa GST•Tag/His•Tag fusion protein were grown at 30 °C, induced and harvested at OD_{600} of 1.2. Equal volumes of cells were aliquoted and pelleted. Pellets were resuspended in respective extraction reagents supplemented with protease inhibitors. The YeastBuster™ Reagent also included 0.01 volume 100X THP Solution. After initial resuspension by pipetting, samples were agitated at room temperature for 20 min. For glass bead extraction, pellets were resuspended in lysis buffer and ~50 µL glass beads, and vortexed on high for 4 min with intermittent chilling on ice. Samples were centrifuged at 16,000 × g for 5 min prior to SDS-PAGE. (B) Total protein extracted by the three methods was determined using Non-Interfering Protein Assay™ Kit. GST activity was determined using GST•Tag Assay Kit. β-gal activity was determined using the host expressing *lac*Z. Samples of the extracts were assayed using the BetaRed™ β-Gal Assay Kit. Data reflect the average of duplicate assays.

Ordering Information

Description	Components	Qty/Pk	Catalogue No.
YeastBuster™ Protein Extraction Reagent*	100 mL YeastBuster™ Protein Extraction Reagent 1 mL 100X THP Solution	100 mL	71186-3
	500 mL YeastBuster™ Protein Extraction Reagent 5 mL 100X THP Solution	500 mL	71186-4
0.5 M THP Solution	0.5 M Solution in water, >80% purity by NMR	1 mL	71194-3
	0.5 M Solution in water, >80% purity by NMR	5 x 1 mL	71194-4

*YeastBuster™ extracts are fully compatible with GST•Bind™ and Ni-NTA His•Bind® IMAC purification methods.

For more information visit: www.merckmillipore.com/psp

Benzonase® Nuclease and rLysozyme™ Solution for Extraction

Enhancers of cell lysis and nucleic acid removal

Remove nucleic acids from and reduce viscosity of protein extracts with Benzonase® Nuclease, a genetically engineered endonuclease from *Serratia marcescens*. It eliminates all forms of DNA and RNA (single-stranded, double-stranded, circular or linear) more efficiently than DNase I without affecting proteins. The enzyme is functional over a wide range of conditions and possesses an exceptionally high specific activity. Adding rLysozyme™ Solution, a highly purified recombinant lysozyme, which degrades bacterial cell walls, enhances extraction efficiency, especially for larger proteins. rLysozyme™ Solution exhibits 250 times higher specific activity than chicken egg white lysozyme.

Features & Benefits

- Increase protein extraction efficiency and facilitate downstream processing of protein extracts with the combined activities of rLysozyme™ Solution and Benzonase® Nuclease
- Effectively reduce viscosity and remove nucleic acids from protein solutions
- Effective over a wide range of conditions and has an exceptionally high specific activity
- Convenient: Available in two purity grades (ultrapure >99% and pure >90%) and in two concentrations (standard at 25 U/µL and high concentration, HC, 250 U/µL)
- Versatile: Compatible with a variety of lysis reagents such as BugBuster® and CytoBuster™ reagents to eliminate viscosity and increase protein yields

Applications

Elimination of Nucleic Acids and Viscosity From Recombinant Proteins, Enhanced Protein Purification, Increased Gel Resolution, Prevention of Cell Clumping

Specifications

Description	Product Details	Concentration	Specific Activity
Benzonase® Nuclease, purity >99%	Effective viscosity reduction and removal of nucleic acids from protein solutions	25 U/µL	1 x 10^6 units/mg protein
Benzonase® Nuclease HC, purity >99%		250 U/µL	1 x 10^6 units/mg protein
Benzonase® Nuclease, purity >90%		25 U/µL	1 x 10^6 units/mg protein
Benzonase® Nuclease HC, purity >90%		250 U/µL	1 x 10^6 units/mg protein
rLysozyme™ Solution	Stabilized recombinant lysozyme	30 KU/µL	250X greater than chicken egg white lysozyme
Chicken Egg White Lysozyme Solution	Ready-to-use, stabilized lysozyme solution	10 mg/mL	
Lysonase™ Bio-Processing Reagent	Convenient blend of rLysozyme™ Solution and Benzonase® Nuclease		

Ordering Information

Description	Qty/Pk	Catalogue No.
Benzonase® Nuclease, purity >99%	10 KU	70664-3
Benzonase® Nuclease HC, purity >99%	25 KU	71206-3
Benzonase® Nuclease, purity >90%	10 KU	70746-3
	2.5 KU	70746-4
Benzonase® Nuclease HC, purity >90%	25 KU	71205-3
rLysozyme™ Solution	300 KU	71110-3
	1200 KU	71110-4
	6000 KU	71110-5
Chicken Egg White Lysozyme Solution	10 x 1 mL	71412-3
Lysonase™ BioProcessing Reagent	0.2 mL	71230-3
	1 mL	71230-4
	5 x 1 mL	71230-5

Note: Benzonase® Nuclease is available in bulk quantities. Please inquire.

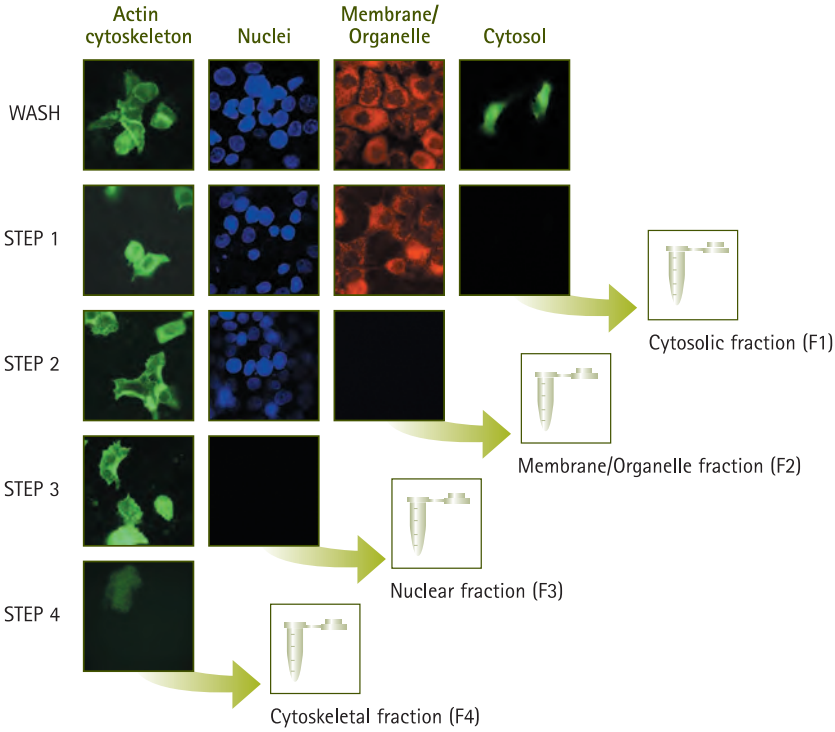
For more information visit: www.merckmillipore.com/psp

ProteoExtract® and ProteoEnrich™ Kits

Sample preparation for proteomics

The ProteoExtract® and ProteoEnrich™ kits cover the different steps in proteomics sample preparation, from protein extraction and abundant protein removal to concentration of protein mixtures, removal of interfering substances, digestion of proteins, selective capturing of phosphorylated peptides, and selective enrichment for specific protein classes. All kits are compatible with each other. Many kits are designed to produce samples that can be used directly in applications such as activity assays, protein microarrays, SDS-PAGE, immunoblotting, ELISA, 2D gel electrophoresis, mass spectrometry (MS; including MS/MS, LC-MS, MALDI-MS, SELDI-MS, and ESI-MS), and others.

Product Performance



Four distinct protein fractions separated using S-PEK. A431 cells were incubated with DAPI (nuclei), phallicidin (to stain actin) and MitoTracker™, extracted and monitored by fluorescence microscopy. These results show that the sequential extraction results in a stepwise degradation of the cell's structure yielding 4 subcellular fractions. In cases where a loss of signal was observed following the extraction, phase contrast images were recorded of the identical field to prove that cells or cell remnants were still present.

Features & Benefits

- Efficient and reproducible protein extraction
- Protease inhibitor cocktail improves results in 2D gel electrophoresis
- Better spot resolution facilitated by nucleic acid digestion with protease-free Benzonase® nuclease
- Designed for compatibility with many applications including activity assays, Western blots, 1D and 2D PAGE, and mass spectrometry
- Optimized protocols for different biological samples

Applications

Cell Fractionation and Organelle Isolation, Membrane Protein Extraction, Subcellular Protein Fractionation, Cytosol/Mitochondria Protein Fractionation, Cytoskeleton Enrichment, Enhancing Resolution of Low-Abundance Proteins, Abundant Protein Removal, Albumin/IgG Depletion, Glycopeptide and Phosphopeptide Enrichment

Ordering Information

Description	Applications	Qty/Pk	Catalogue No.
ProteoExtract® Subcellular Protein Extraction Kit	Organelle Fractionation	20 reactions	539790
ProteoExtract® S-PEK Antibody Control Kit	Organelle Fractionation	1 kit	71771-3
ProteoExtract® Complete Mammalian Protein Extraction Kit	Organelle Fractionation	20 reactions	539779
ProteoExtract® Cytosol/Mitochondria Fractionation Kit	Organelle Fractionation	100 extractions	QIA88
ProteoExtract® Native Cytoskeleton Enrichment Kit	Organelle Fractionation	32 assays	17-10210
ProteoExtract® Cytoskeleton Enrichment and Isolation Kit	Organelle Fractionation	15 reactions	17-10195
ProteoExtract® Native Membrane Protein Extraction Kit	Membrane Proteins	20 reactions	444810
ProteoExtract® Transmembrane Protein Extraction Kit	Membrane Proteins	20 reactions	71772
ProteoExtract® All-in-One Trypsin Digestion Kit	Mass Spec Peptide Enrichment	100 digests	650212

Ordering Information – Continued

Description	Applications	Qty/Pk	Catalogue No.
ProteoExtract® Glycopeptide Enrichment Kit	Mass Spec Peptide Enrichment	50 enrichment reactions	72103
ProteoExtract® Phosphopeptide Enrichment TiO_2 Kit	Mass Spec Peptide Enrichment	100 reactions	539722
ProteoEnrich® CAT-X Kit	Mass Spec Peptide Enrichment	2 cartridges; each resuable up to 10x	71532-3
ProteoExtract® Albumin Removal Kit	Albumin and IgG Depletion	12 samples	122640
ProteoExtract® Albumin Removal Kit Maxi	Albumin and IgG Depletion	20 samples	122641
ProteoExtract® Albumin/IgG Removal Kit	Albumin and IgG Depletion	12 samples	122642
ProteoExtract® Albumin/IgG Removal Kit Maxi	Albumin and IgG Depletion	20 samples	122643
ProteoExtract® Tissue Dissociation Buffer Kit	Other	10 reactions	539720
ProteoExtract® Collagenase Set	Other	1 kit	71777-3
ProteoExtract® Protein Precipitation Kit	Other	200 precipitations	539180

For more information visit: www.merckmillipore.com/psp

Calbiochem® Protease and Phosphatase Inhibitor Cocktails

Pre-mixed cocktails preserve protein sample integrity

Protease and phosphatase inhibitors are essential for maintenance of expressed proteins and subsequently for their characterization, biomarker discovery, mapping of post-translational modifications and protein quantification. Calbiochem® protease and phosphatase inhibitor cocktails are provided as ready-to-use, no-waste liquid stock solutions with complete formulation details. Choose the product that's designed for your specific application.

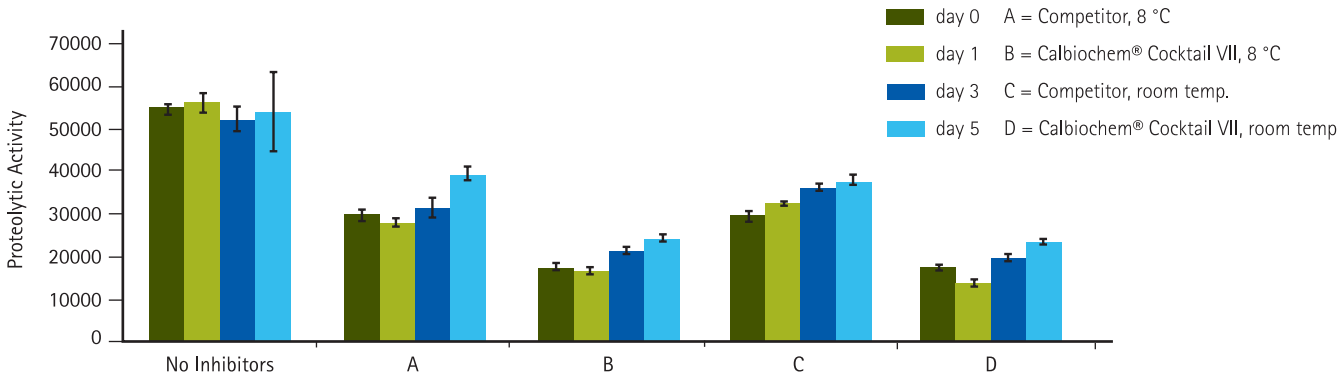
Features & Benefits

- Ready-to-use, waste-free liquid formulations for greater convenience
- Stringent quality control for reproducibility and excellent inhibition over a wide range of protease classes
- Full disclosure of formulations and a comprehensive selection of specific cocktails optimized for most tissue or cell type extracts ensures greater experimental flexibility

Applications

Protein Sample Preparation and Preservation, Signal Transduction Studies, Western Blotting

Product Performance



Increased efficiency and stability of Calbiochem® protease inhibitor dilutions compared to a competitor tablet. Protease inhibitors (Competitor or Calbiochem® Cocktail VII, Catalogue No. 539138)were diluted to their working concentration in BugBuster® lysis reagent.Addition of protease inhibitor cocktails inhibits the protease PRONASE®(Cat. No. 537088), resulting in reduced fluorescence when measured using the Universal HT Protease Assay. On day 1, for samples incubated at 8 °C, the competitor tablet inhibited the proteolytic activity by 50% and the Calbiochem® Cocktail VII inhibited it by 70%. On day 5, for samples incubated at 8 °C, the competitor tablet caused a 29% decrease in proteolytic activity in comparison to the Calbiochem® cocktail VII, which caused a 57% decrease.

Ordering Information

Description	Recommended Applications	Catalogue No.
Protease Inhibitor Cocktail Set I	General use	539131
Protease Inhibitor Cocktail Set II	Bacterial cell extracts (except those intended for metal chelation chromatography)	539132
Protease Inhibitor Cocktail Set III, EDTA-Free	Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2D gel electrophoresis	539134
Protease Inhibitor Cocktail Set IV	Fungal and yeast cell extracts	539136
Protease Inhibitor Cocktail Set V, EDTA-Free	Mammalian cells and tissue extracts purified using metal chelation chromatography; samples to be analyzed by 2D gel electrophoresis	539137
Protease Inhibitor Cocktail Set VI	Plant cell extracts	539133
Protease Inhibitor Cocktail Set VII	Proteins containing His•Tag® sequences	539138
Serine Protease Inhibitor Cocktail Set I	Broad-range serine protease inhibition	565000
Phosphatase Inhibitor Cocktail Set I	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A	524624
Phosphatase Inhibitor Cocktail Set II	Protection against acid and alkaline phosphatases and Protein Tyrosine Phosphatases (PTPs)	524625
Phosphatase Inhibitor Cocktail Set III	Protection against acid, alkaline and Ser/Thr phosphatases and PTPs	524627
Phosphatase Inhibitor Cocktail Set IV	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A	524628
PhosphoSafe™ Extraction Reagent	Protection against Ser/Thr phosphatases and PTPs	71296

For more information visit: www.merckmillipore.com/inhibitors

Calbiochem® Buffers and Detergents for Protein Extraction

Don't just rely on good fortune, use high quality products you can trust

Benefit from a wide selection of Calbiochem® buffers and detergents, each tailored for specific applications. For greater flexibility some buffers and detergents can be purchased either as solids or as ready-to-use solutions.

Features & Benefits

- Wide selection of buffers and detergents tailored for specific applications
- Stringent quality testing to ensure lot-to-lot consistency
- Suitable for research labs, as well as production facilities

Applications

Protein Sample Preparation, Western Blotting

Ordering Information

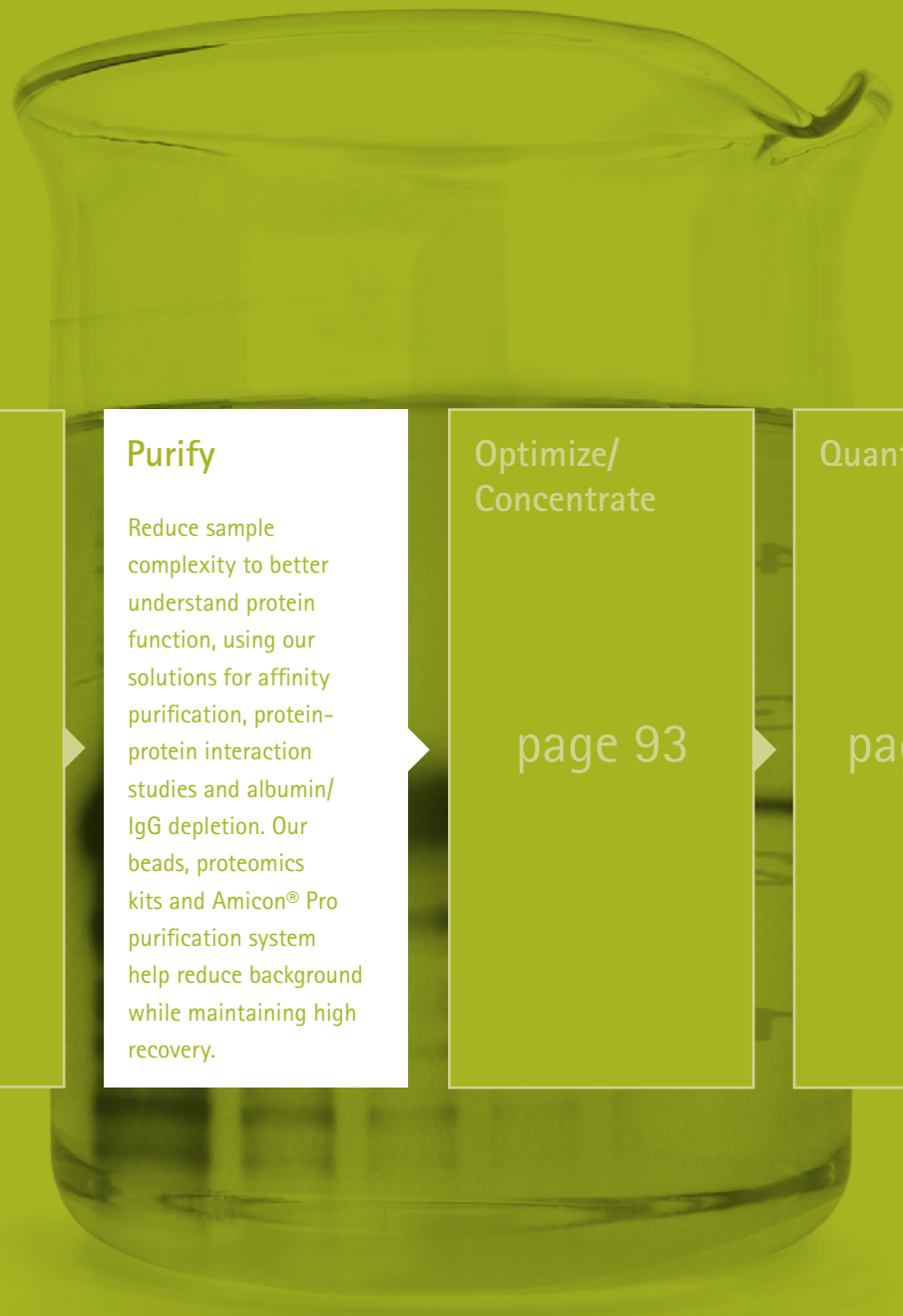
Description	Qty/Pk	Catalogue No.
Calbiochem® Buffers		
Triethylammonium Acetate, 1M solution	1 L	625718
HEPES, free acid, ULTROL® grade solution	25 g, 100 g, 500 g, 1 kg, 5 kg	391338
PBS Tablets	10 tablets	524650
Calbiochem® Detergents		
ZWITTERGENT® 3-14 Detergent	5 g, 25 g, 100 g, 500 g	693017
Digitonin, high purity	250 mg, 1 g, 5 g	300410
CHAPS	1 g, 5 g, 10 g, 25 g, 250 g, 1 kg	220201

Note: Visit our website for a complete product listing.

For more information visit: www.merckmillipore.com/biochemicals


50,000
NMWCO
µL
1500
1000
500
250

Protein Sample Preparation



Extract

page 67

Purify

Reduce sample complexity to better understand protein function, using our solutions for affinity purification, protein-protein interaction studies and albumin/IgG depletion. Our beads, proteomics kits and Amicon® Pro purification system help reduce background while maintaining high recovery.

Optimize/ Concentrate

page 93

Quantify/Detect

page 111

Amicon® Pro Purification System

Purify, exchange buffer and concentrate in one device

Traditional protein purification is a long process with many steps and multiple devices. Avoid the risks associated with sample transfer and reduce hands-on time when you bind, wash, elute and/or concentrate your protein in the all-in-one Amicon® Pro purification system. The device combines affinity-based spin column purification with downstream sample concentration and buffer exchange. Featuring a large reservoir that accommodates a range of sample volumes, the device reduces the need for multiple centrifugation steps. Simply attach the included Amicon® Ultra filter for simultaneous elution, concentration and highly efficient buffer exchange (>99%) in a single spin.

Features & Benefits

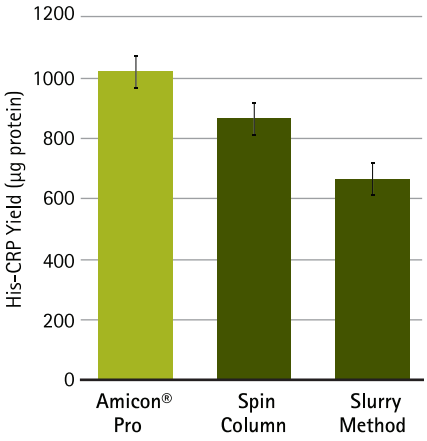
- High yield: No sample transfer means no sample loss
- Gentle: Novel design enables gentle, continuous flow for efficient buffer exchange
- Fast: Go from lysate to purified protein, in buffer of your choice, in just 5 spins
- Flexible: Configure the modular device to fit a range of sample prep needs

Applications

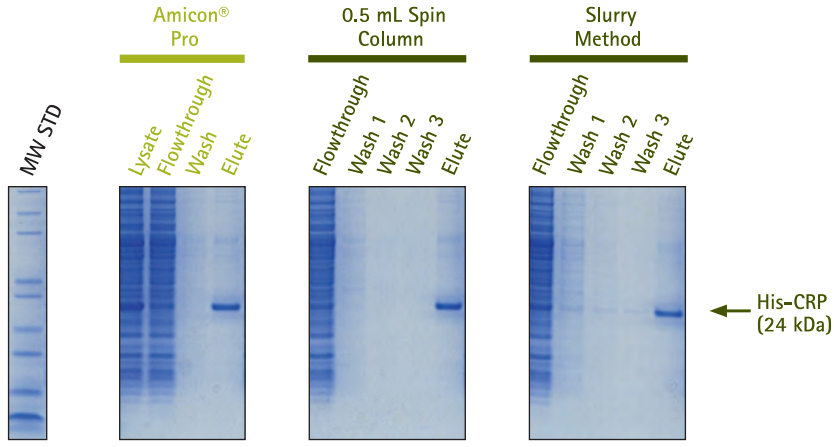
Affinity Purification, Depletion of Abundant Proteins, Protein Enrichment, Buffer Exchange, Desalting, Clean-up of Antibody Labeling Reactions

Product Performance

A.



B.



Better yield, equal protein purity from Amicon® Pro purification compared to two traditional affinity purification schemes. In each case, 100 µL settled resin was used to purify His-CRP from 0.5 mL *E coli* lysate. (A) The graph shows the difference in total protein yield (His-CRP) from the three different bind-wash-elute methods. Bars represent the average of 12 (Amicon® Pro), 6 (0.5 mL spin column), or 4 (slurry method) independent tests. (B) A representative gel showing the various fractions derived during purification using the three methods being compared.

Specifications

Amicon® Pro Application	Protocol Steps	Maximum Capacity	Benefits[4]
Purification with Buffer Exchange and/ or Concentration	Bind Clear and Wash Elute/Concentrate +/- Buffer Exchange	200 µL packed resin[1]	Speed No sample transfer - No loss Improved yield
Purification Only	Bind Clear and Wash Elute	1000 µL packed resin[2]	Range of sample volumes can be processed
Buffer Exchange	Buffer Exchange +/- Concentrate	Variable input[3]	Speed Improved activity of purified protein
Antibody Labeling	Buffer Exchange Labeling Reaction Wash and Concentrate +/- Buffer Exchange	Variable antibody input[3]	Speed No sample transfer - No loss Improved yield Read application note
Depletion or Enrichment	Bind Deplete/Concentrate +/- Wash/Concentrate +/- Buffer Exchange	200 µL packed resin[1]	Speed No sample transfer - No loss

[1] The assay capacity is dictated by the processing limitations of the Amicon® Ultra 0.5 mL device.

[2] The Bind-Wash-Elute protocol is linearly scalable (50-1000 µL).

[3] Capacity depends on diafiltration centrifugation protocol.

[4] Benefits are relative to other current methods.

Ordering Information

Description	MWCO	Qty/Pk	Catalogue No.
Amicon® Pro Purification Kits			
Amicon® Pro Affinity Concentration Kit Ni-NTA	3,000	12	ACK5003NT
	10,000	12	ACK5010NT
	30,000	12	ACK5030NT
	50,000	12	ACK5050NT
	100,000	12	ACK5100NT
Amicon® Pro Affinity Concentration Kit Protein A	3,000	12	ACK5003PA
	10,000	12	ACK5010PA
	30,000	12	ACK5030PA
	50,000	12	ACK5050PA
	100,000	12	ACK5100PA
Amicon® Pro Affinity Concentration Kit Protein G	3,000	12	ACK5003PG
	10,000	12	ACK5010PG
	30,000	12	ACK5030PG
	50,000	12	ACK5050PG
	100,000	12	ACK5100PG
Amicon® Pro Affinity Concentration Kit GST	3,000	12	ACK5003GS
	10,000	12	ACK5010GS
	30,000	12	ACK5030GS
	50,000	12	ACK5050GS
	100,000	12	ACK5100GS

Description	MWCO	Qty/Pk	Catalogue No.
Amicon® Pro Purification System – No Reagents Included			
Amicon® Pro Purification System	3,000	12	ACS500312
		24	ACS500324
	10,000	12	ACS501012
		24	ACS501024
	30,000	12	ACS503012
		24	ACS503024
	50,000	12	ACS505012
		24	ACS505024
	100,000	12	ACS510012
		24	ACS510024
Amicon® Pro Purification System – No Filters Included			
Amicon® Pro Purification System (excluding filter)	N/A	24	ACS500024
Reagent Kit Only			
Ni-NTA Reagent Kit	N/A	1	ACR5000NT
Protein A Reagent Kit	N/A	1	ACR5000PA
Protein G Reagent Kit	N/A	1	ACR5000PG
GST Reagent Kit	N/A	1	ACR5000GS

For more information visit: **www.merckmillipore.com/amiconpro**

Simultaneous lysis and capture using the Amicon® Pro system expedites purification of bacterially expressed recombinant proteins

The standard protein purification workflow involves extraction, affinity-based capture, and sample optimization (Figure 1). Traditional mechanical lysis methods are tedious and harsh, leading to diminished protein integrity and prep-to-prep variability. Gravity-driven agarose columns are frequently used for affinity purification. While easy to manipulate, columns may clog due to debris or high lysate viscosity, so necessitating the clarification of the lysate before it is added to the column. Moreover, final optimization of buffer composition and protein concentration requires a separate device, increasing the risk of sample loss. Here, we demonstrate a condensed workflow, combining the bacterial cell lysis and affinity capture steps (Figure 1). This is made possible by the gentle, detergent-based BugBuster® Master Mix lysis reagent, which includes Benzonase® nuclease for reducing lysate viscosity, and the Amicon® Pro purification system, which directly links affinity-based spin column purification with buffer exchange and concentration.

Following resuspension of bacterial cell pellets in BugBuster® Master Mix, all steps were performed within the Amicon® Pro device. Cell pellets were converted to purified, concentrated protein in the correct buffer for downstream application. This workflow requires fewer process steps and less total time without sacrificing yield or sample purity (Figures 2 and 3). Confining the sample to a single device reduces loss and minimizes inter-prep variation.

For Amicon® Pro System ordering information, see page 80.
For BugBuster® Master Mix ordering information, see page 71.

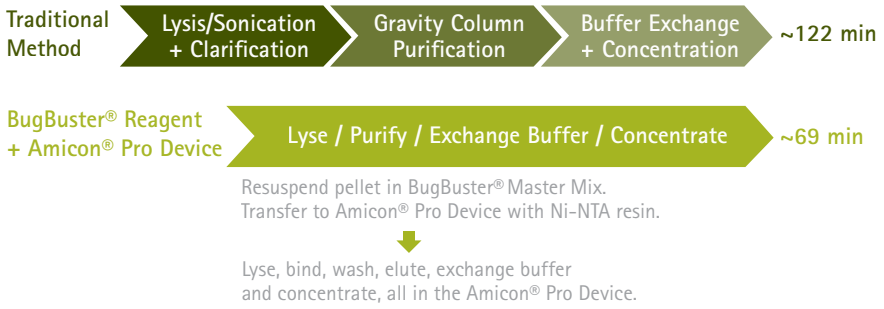


Figure 1. Condensing the recombinant protein purification workflow. Compared to traditional purification involving mechanical lysis (sonication), affinity purification using gravity columns, plus additional devices for final sample formulation, less time is required for combined chemical lysis/capture/sample optimization, using BugBuster® Master Mix and the Amicon® Pro system.

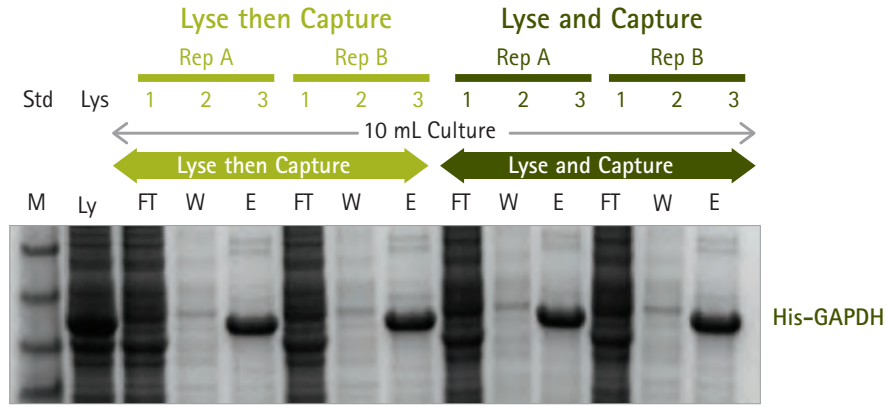


Figure 2. Combined lysis and capture maintains protein yield and purity. For "Lyse then Capture", a cell pellet was resuspended in BugBuster® Master Mix, lysed by agitation, clarified by centrifugation and purified in the Amicon® Pro device. For "Lyse and Capture," a replicate pellet was resuspended in BugBuster® Master Mix and then mixed with resin in the Amicon® Pro device without clarification. Fractions were analyzed by SDS-PAGE. **Lanes:** Std - molecular weight standard, Lys - lysate pre-passage, 1 - flowthrough, 2 – wash fraction, and 3 – elution fraction.

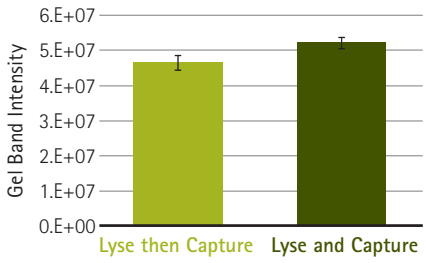


Figure 3. Relative protein recovery in the respective elution fractions was determined by gel densitometry. For each method, the bar represents the average of four individual replicate purifications. On average, for a 10 mL pellet, "Lyse and Capture" resulted in approximately 10% greater his-GAPDH yield than the "Lyse then Capture" method.

Agarose-Based Affinity Purification

IP and antibody purification

Protein A and Protein G are proteins of microbial origin that bind specifically but differently to mammalian immunoglobulins. When coupled to agarose, they provide an efficient tool for purification and immunoprecipitation (IP) of antibodies. Protein A agarose binds to the Fc region of IgG from a variety of species and can be used to purify classes, subclasses, and fragments of Igs and to isolate immune complexes. Protein G agarose is useful for binding to Igs that do not bind Protein A and can be used for antibody IP for purifying Igs and IgG fractions. Combining Protein A and Protein G agarose is a good strategy for exploiting the power of both Ig binding affinities in a single reagent.

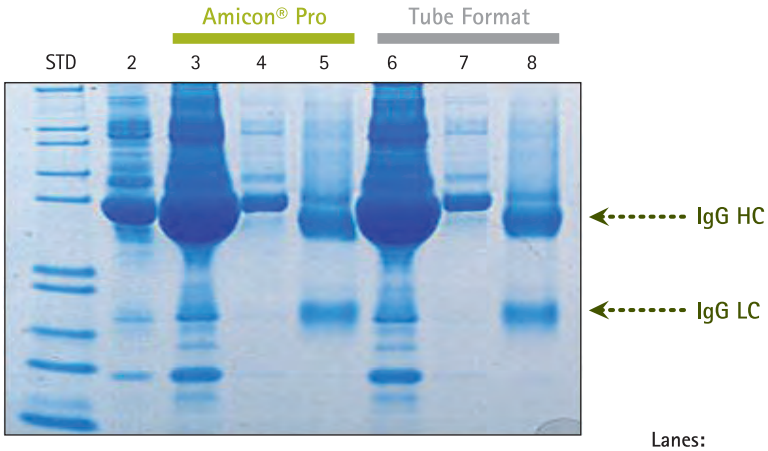
Features & Benefits

- Agarose beads with either immobilized Protein A, Protein G, or a mixture of Protein A and G for optimal affinity
- Compatible with Amicon® Pro purification system
- High binding capacity and low ligand leaching
- Two convenient formats, one for purification in column or batch mode and the other is a ready to-use suspension containing BSA for IP applications

Applications

Immunoprecipitation, Affinity Purification, Antibody Purification

Product Performance



100 µL Protein A beads were mixed with 1 mL rabbit serum (1:10 diluted) and IgG was purified by standard tube protocol or using an Amicon® Pro device. IgG purification was near complete using either method.

Lanes:
2: Serum,
3 and 6: Flowthrough
4 and 7: Wash fraction
5 and 8: Eluted fraction

Ordering Information

Description	Product Details	Qty/Pk	Catalogue No.
Protein A			
Protein A Agarose Suspension	Pre-blocked for IP	1.5 mL	IP02-1.5ML
Protein A Agarose	Purification of mouse IgG_{2a} and IgG_{2b}	10 mL	16-125
Protein A Agarose Fast Flow	Purification of mouse IgG_{2a} and IgG_{2b}	10 mL	16-156
Protein G			
Protein G Agarose Fast Flow	Purification of mouse IgG_1 and IgG_3 and rat IgG	10 mL	16-266
Protein G Plus Agarose Suspension	Pre-blocked for IP	1.5 mL	IP04-1.5ML
Protein A/G Mix			
Protein G Plus/Protein A Suspension	Pre-blocked for IP	1.5 mL	IP05-1.5ML
Protein G Plus/Protein A	Purification of mouse IgG and rat IgG	10 mL	IP10-10ML
Montage® Antibody Purification Kits			
Montage® Antibody Purification Kit with PROSEP®-A Media	High capacity pre-packed spin columns	20 purifications	LSK2ABA20
Montage® Antibody Purification Kit with PROSEP®-G Media	High capacity pre-packed spin columns	20 purifications	LSK2ABG20

For more information visit: www.merckmillipore.com/psp

Agarose Selector Guide

Description	Size	Recommended for Amicon® Pro System	Supplied As:	Binding Capacity	Application	Cat No.
Protein A Agarose Suspension	1.5 mL	+	30% slurry	Use 15 µL suspension/ µg antibody	IP: pre-blocked with BSA. Not for purification	IP02-1.5ML
Protein A Agarose	10 mL	++	50% slurry	20 ± 2 mg human IgG/mL settled agarose	IP; affinity purification	16-125
Protein A Agarose Fast Flow	10 mL	+	50% slurry; highly cross-linked 6% agarose beads	40 mg human IgG/mL agarose	Medium and low pressure chromatography (flow rates 50 - 300 cm/h)	16-156
Protein G Agarose Fast Flow	10 mL	++	50% slurry; highly cross-linked 4% agarose beads	20 mg human IgG/mL agarose	Medium and low pressure chromatography (flow rates 50 - 300 cm/h)	16-266
Protein G Plus Agarose Suspension	1.5 mL	+	30% slurry	Use 15 µL suspension/ µg antibody	IP: pre-blocked with BSA. Not for purification	IP04-1.5ML
Protein G Plus/ Protein A Suspension	1.5 mL	+	30% slurry	Use 15 µL suspension/ µg antibody	IP: pre-blocked with BSA. Not for purification	IP05-1.5ML
Protein G Plus/ Protein A Agarose	10 mL	+	50% slurry	Use 5-10 mL of packed beads per mL serum	Antibody purification	IP10-10ML
Streptavidin Agarose	10 mL	+	50% slurry	1.5 to 2.5 mg/mL of biotinylated rabbit IgG	IP; column or batch purification of biotinylated molecules	16-126
Streptavidin Agarose	5 mL	+	50% slurry	> 85 nmol free biotin/mL	IP; column or batch purification of biotinylated molecules	69203-3
His•Bind® Resin	10 mL	+	Uncharged IDA agarose resin	8 mg/mL bed volume	Uncharged resin: User can charge with metal ion of choice. Small to medium scale purifications using either gravity flow columns or batch method	69670-3
	50 mL	+				69670-4
	100 mL	+				69670-5
His•Bind® Buffer Kit	1 kit	+	**Separate vials of:** Bind, Wash, Elution, Stripping, and Charging Buffers	n/a	Solutions are included for Ni^{2+} charging, binding, washing and elution of up to ten 2.5 mL columns	69755-3
His•Bind® Purification Kit	1 kit	(comes with chromatography columns)	• 10 mL His•Bind® Resin • 1 His•Bind® Buffer Kit • pkg/4 Chromatography Columns	8 mg/mL bed volume	Small scale purifications using gravity flow columns	70239-3
Ni-NTA His•Bind® Resin	10 mL	++	50% slurry	5–10 mg His•Tag® fusion protein per mL resin	One-step gravity flow purification of proteins containing a His•Tag® sequence	70666-3
	25 mL	++				70666-4
	100 mL	++				70666-5
Ni-NTA His•Bind® Superflow™ Resin	10 mL	+	50% slurry		Compatible with FPLC	70691-3
	25 mL	+				70691-4
	100 mL	+				70691-5
BugBuster® Ni-NTA His•Bind® Purification Kit	1 kit	(comes with chromatography columns)	**Separate vials of**: BugBuster® Protein Extraction Reagent; Benzonase® Nuclease, purity >90%; Ni-NTA His•Bind® Resin; Chromatography Columns	5–10 mg His•Tag® fusion protein per mL resin	Gentle lysis of *E. coli* to release soluble protein and one-step gravity flow purification of proteins containing a His•Tag® sequence	70751-3
BugBuster® His•Bind® Purification Kit	1 kit	(comes with chromatography columns)	**Separate vials of**: BugBuster® Protein Extraction Reagent; Benzonase® Nuclease, Purity >90%; His•Bind® Resin; His•Bind® Buffer Kit; Chromatography Columns	8 mg/mL bed volume	Gentle lysis of *E. coli* to release soluble protein and one-step gravity flow purification of proteins containing a His•Tag® sequence	70793-3
Ni-NTA Buffer Kit	1 kit	++	**Separate vials of**: Ni-NTA Bind Buffer, Ni-NTA Wash Buffer, Ni-NTA Elute Buffer	n/a	Set of buffers optimized for purification of His•Tag® fusion proteins on Ni-NTA His•Bind® Resin. These phosphate-buffered solutions differ from the Tris-based solutions used in the His•Bind® Buffer Kit	70899-3

■ Tested
■ Recommended, but not tested

Agarose Selector Guide

Description	Size	Recommended for Amicon® Pro System	Supplied As:	Binding Capacity	Application	Cat No.
GST•Bind™ Resin	10 mL	++	50% slurry	5–8 mg GST•Tag™ fusion protein per 1 mL settled resin	Column or batch format purification of recombinant glutathione S-transferase (GST) fusion proteins or native glutathione S-transferase or glutathione-binding proteins	70541-3
	50 mL	++				70541-4
	25 mL	++				70541-5
BugBuster® GST•Bind™ Purification Kit	1 kit	(comes with chromatography columns)	**Separate vials of**: BugBuster® Protein Extraction Reagent; Benzonase® Nuclease; GST•Bind™ Resin; Chromatography Columns; GST Bind/Wash and Reconsititution Buffers Glutathione, Reduced		Gentle lysis of *E. coli* to release soluble protein and purification of recombinant glutathione S-transferase (GST) fusion proteins or native glutathione S-transferase or glutathione-binding proteins	70794-3
GST•Bind™ Buffer Kit	1 kit	++	**Separate vials of**: GST Bind/Wash Buffer; Glutathione Reconstitution Buffer; Glutathione, Reduced	n/a	Set of pretested buffers for binding, washing and elution of GST•Tag fusion proteins from GST•Bind™ Resin or GST•Mag™ Agarose Beads	70534-3
Strep•Tactin Superflow Agarose	2 mL	+	50% slurry	50-100 nmol/mL settled resin, or up to 3 mg of 30 kDa protein per mL settled resin	Low pressure and FPLC chromatography	71592-3
	10 mL	+				71592-4
Strep•Tactin Buffer Kit	1 kit	+	**Separate vials of**: Strep•Tactin Wash, Elution, and Regeneration Buffers	n/a	Pretested buffers for use with Strep•Tactin Resins to purity Strep•Tag® II fusion proteins	71613-3
Strep•Tactin SpinPrep™ Kit	1 kit	(prepacked mini spin columns)	• 25 Strep•Tactin SpinPrep™ Columns and Collection Tubes • Separate vials of Strep•Tactin Wash Buffer and Elution Buffer	Each column purifies up to 150 µg of Strep•Tag® II fusion protein	Purification of Strep•Tag® II fusion proteins using mini spin columns	71608-3
D-Desthiobiotin	1 g	+	lyophilized powder	n/a	Gentle elution of Strep•Tag® II proteins from the biotin-binding site of Strep•Tactin® resins	71610-3
T7•Tag® Affinity Purification Kit	1 kit	(comes with chromatography columns)	**Separate vials of**: T7•Tag® Antibody Agarose; T7•Tag® Bind/Wash Buffer, Elution Buffer, and Neutralization Buffer; Chromatography Column	> 300 µg T7•Tag® β-galactosidase per mL of settled resin	Rapid immunoaffinity purification of target proteins that carry the T7•Tag® sequence (i.e., the amino terminal 11 aa of the T7 gene 10 protein)	69025-3
T7•Tag® Antibody Agarose	2 mL	+	50% slurry			69026-3
S-Protein Agarose	2 mL	+	50% slurry	The capacity varies and is based on the size and folding characteristics of a given target protein	Purification of S•Tag™ fusion proteins	69704-3
	10 mL	+				69704-4
S•Tag™ Thrombin Purification Kit	1 kit	(comes with spin columns)	**Separate vials of**: S-protein Agarose; Bind/Wash Buffer; Thrombin Cleavage Buffer; Biotinylated Thrombin; Streptavidin Agarose; Spin Filters			69232-3
S•Tag™ rEK Purification Kit	1 kit	(comes with spin columns)	**Separate vials of**: S-protein Agarose; Bind/Wash Buffer; rEK Dilution/Storage Buffer; Recombinant Enterokinase; EKapture™ Agarose; Spin Filters			69065-3

■ Tested
■ Recommended, but not tested

Agarose Purification of Recombinant Fusion Proteins

His•Tag®, GST•Tag, S•Tag™, Strep•Tag II, and T7•Tag®

For recombinant proteins, the addition of fusion tags using appropriate expression vectors enables affinity purification by a number of strategies. Here we showcase products specifically designed for the rapid purification of fusion proteins containing His•Tag®, GST•Tag, S•Tag™, Strep•Tag II, and T7•Tag® sequences. These products are optimized for purification of proteins expressed in bacterial, yeast, insect, or mammalian systems. Reagents and kits are available in a variety of configurations, providing convenient options.

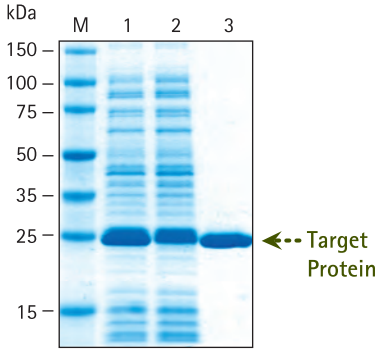
Features & Benefits

- Resins available for purification of a variety of fusion tags
- Compatible with Amicon® Pro purification system
- Optimized for purification of proteins expressed in bacterial, yeast, insect, or mammalian systems
- Convenient variety of kits and resin formats available
- Premium quality fusion tag monoclonal antibodies and Western blot kits also available

Applications

Affinity Purification of Tagged Recombinant Fusion Proteins

Product Performance



Lane M: Marker
Lane 1: BugBuster® Extract
Lane 2: Flowthrough
Lane 3: Eluate

GST•Bind™ purification. A crude extract containing unfused GST was applied to a 2 mL GST•Bind™ Resin column. Total protein yield after purification was 8 mg/mL resin.

Ordering Information

Description	Product Details	Qty/Pk	Catalogue No.
His-Tag Purification			
His•Bind® Resin	Easily charged with metal ion of choice. Reusable many times. Compatible with His•Bind® Buffer Kit. Compatible with 1 mM THP	10 mL	69670-3
		50 mL	69670-4
		100 mL	69670-5
His•Bind® Buffer Kit	Solutions for up to ten 2.5-mL columns	1 kit	69755-3
His•Bind® Purification Kit	Contains resin and buffers for small scale purification using gravity-flow columns	1 kit	70239-3
BugBuster® His•Bind® Purification Kit	Convenient preparation of extracts and affinity purification of His•Tag® fusion proteins	1 kit	70793-3
Ni-NTA His•Bind® Resin	Minimal Ni^{2+} leaching. Compatible with 20 mM 2-ME and 1 mM THP. Compatible with Ni-NTA Buffer Kit	10 mL	70666-3
		25 mL	70666-4
		100 mL	70666-5
Ni-NTA His•Bind® Superflow™ Resin	Minimal Ni^{2+} leaching. Compatible with 20 mM 2-ME and 1 mM THP. Compatible with Ni-NTA Buffer Kit. High flow rates and pressures	10 mL	70691-3
		25 mL	70691-4
		100 mL	70691-5
Ni-NTA Buffer Kit	Set of 4X pretested buffers compatible with Ni-NTA His•Bind® resins	1 kit	70899-3
BugBuster® Ni-NTA His•Bind® Purification Kit	Convenient preparation of extracts and affinity purification of His•Tag® fusion proteins	1 kit	70751-3

Ordering Information – Continued

Description	Product Details	Qty/Pk	Catalogue No.
GST-Tag Purification			
GST•Bind™ Resin	Can be reused up to 6 times without loss of capacity	10 mL	70541-3
		50 mL	70541-4
		25 mL	70541-5
BugBuster® GST•Bind™ Purification Kit	Convenient preparation of soluble extracts and affinity purification of GST•Tag fusion proteins	1 kit	70794-3
GST•Bind™ Buffer Kit	Sufficient components for up to ten 2.5-mL GST•Bind™ columns	1 kit	70534-3
Strep-Tag II Purification			
Strep•Tactin Superflow Agarose	Resuable 3-6 times. Compatible with 1 M urea or guanidine, 2% Triton® X-100, 0.1% SDS, and 50 mM DTT or 2-ME	2 mL	71592-3
		10 mL	71592-4
Strep•Tactin Buffer Kit	Set of 10X pretested buffers for use with Strep•Tactin resins	1 kit	71613-3
Strep•Tactin SpinPrep™ Kit	Compatible with 1 M urea or guanidine, 2% Triton® X-100, 0.1% SDS, and 50 mM DTT or 2-ME	1 kit	71608-3
D-DESTHIOBIOTIN	Lyophilized powder	1 g	71610-3
T7-Tag Purification			
T7•Tag® Affinity Purification Kit	Buffer included to limit protein exposure to low pH. Beads are reusable >5 times without loss of binding activity	1 kit	69025-3
T7•Tag® Antibody Agarose	Column or batch purification methods. Beads are reusable >5 times without loss of binding activity	2 mL	69026-3
S-Tag Purification			
S-Protein Agarose	Specifically retains S•Tag™ fusion proteins	2 mL	69704-3
		10 mL	69704-4
S•Tag™ Thrombin Purification Kit	Sufficient reagents provided for purification of up to 1 mg target protein under native or denaturing conditions	1 kit	69232-3
S•Tag™ rEK Purification Kit	Sufficient reagents provided for purification of up to 1 mg target protein under native or denaturing conditions	1 kit	69065-3
Streptavidin			
Streptavidin Agarose Conjugate	Routinely evaluated by immunoprecipitating tyrosine-phosphorylated proteins from a RIPA lysate prepared from EGF-stimulated A431 cells with a biotinylated monoclonal phosphotyrosine antibody (Catalogue No. 16-103)	10 mL	16-126
Streptavidin Agarose	No detectable protease, DNase, or RNase contamination	5 mL	69203-3

For more information visit: **www.merckmillipore.com/psp**

PureProteome™ Magnetic Beads

Magnetic bead-based isolation of proteins

For extremely fast and easy protein purification, stick with PureProteome™ magnetic beads. These magnetic bead purification systems feature low, non-specific binding and minimal sample loss. Conventional purification methods require centrifugation to pellet, followed by careful aspiration to avoid losing sample. Magnetic beads are isolated using a magnetic stand, enabling total removal of buffers and complete recovery of beads with no sample loss.

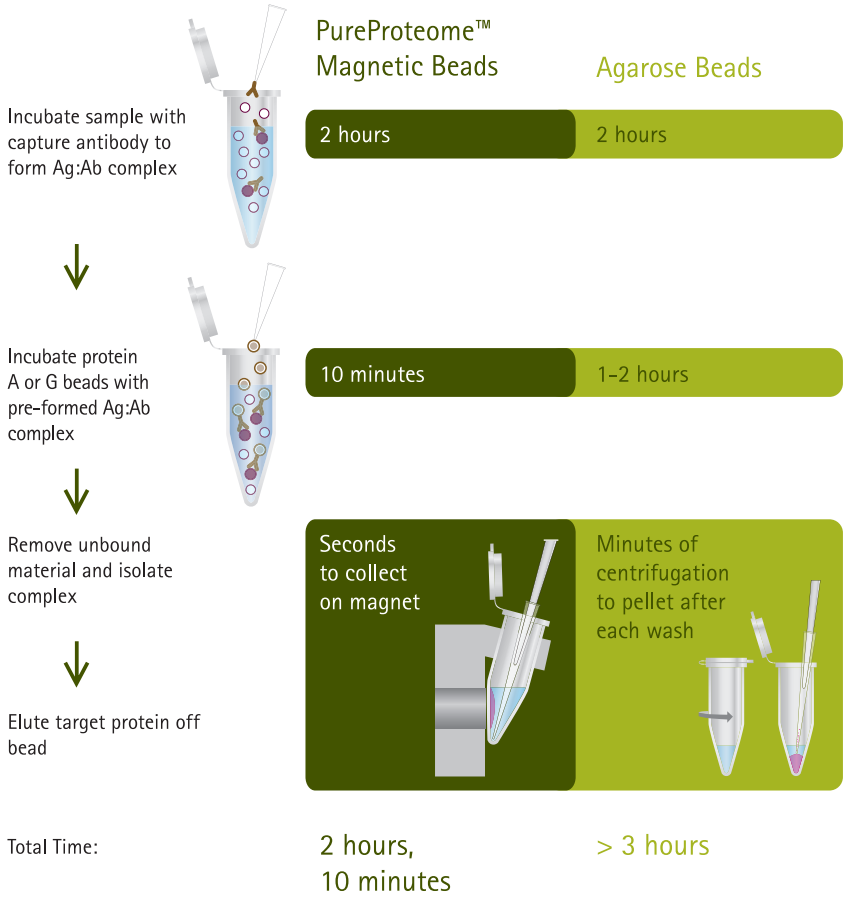
Features & Benefits

- High capacity: Advanced chemistry combined with high surface area provides more binding sites for proteins
- Consistent results with no sample loss: Particles visible as they adhere to side of tube for quick and easy aspiration and complete buffer removal
- Fast processing time: Beads are immobilized in seconds. Increased kinetics of bead-protein binding enables shorter incubations
- Economical: Significantly more affordable than competitive magnetic beads

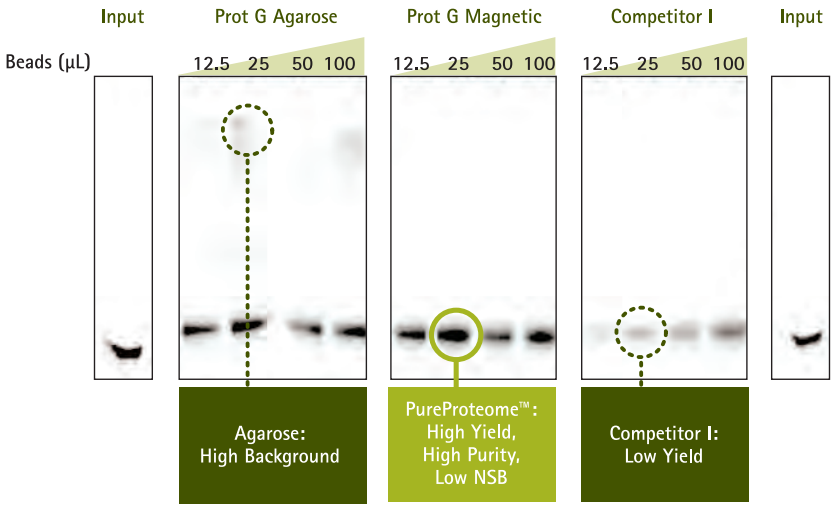
Applications

Immunoprecipitation, Affinity Purification, Recombinant Protein Purification, Biotinylated Molecule Isolation, Depletion/Enrichment, Antibody Purification, Fab Purification

Product Performance



High speed immunoprecipitation with magnetic beads compared to agarose. In parallel indirect immunoprecipitations, PureProteome™ magnetic beads offered a 50% reduction in incubation time while yielding results equivalent to agarose beads.



PureProteome™ Protein G magnetic beads outperform the competition in immunoprecipitation. Compared to traditional agarose beads and Competitor I Protein G magnetic beads, PureProteome™ magnetic beads provide higher protein yield with virtually no background binding. HEK293 cells were transfected with a ~20 kDa protein. Cell lysates were mixed with various volumes of protein G beads and rabbit serum containing antibody specific to the 20 kDa protein. After overnight incubation at 4 °C, beads were washed and resuspended in sample buffer. The bead-bound protein fraction was detected by Western blotting. Data courtesy of University of Washington (Department of Pharmacology) Seattle, WA.

Ordering Information

Description	Qty/Pk	Catalogue No.
Conjugated Beads		
PureProteome™ Protein A Magnetic Bead System	2 x 1 mL	LSKMAGA02
	10 mL	LSKMAGA10
PureProteome™ Protein G Magnetic Bead System	2 x 1 mL	LSKMAGG02
	10 mL	LSKMAGG10
PureProteome™ Protein A/G Mix Magnetic Beads	2 x 1 mL	LSKMAGAG02
	10 mL	LSKMAGAG10
PureProteome™ Albumin Magnetic Beads	10 mL	LSKMAGL10
PureProteome™ Kappa Ig Binder Magnetic Beads	2 x 1 mL	LSKMAGKP02
PureProteome™ Lambda Ig Binder Magnetic Beads	2 x 1 mL	LSKMAGLM02
PureProteome™ Nickel Magnetic Bead System	2 x 1 mL	LSKMAGH02
	10 mL	LSKMAGH10
PureProteome™ Streptavidin Magnetic Bead System	2 x 1 mL	LSKMAGT02
	10 mL	LSKMAGT10
Active Chemistry Beads		
PureProteome™ NHS FlexiBind Magnetic Beads Kit	0.5 mL	LSKMAGN01
PureProteome™ NHS FlexiBind Magnetic Bead System	4 x 0.5 mL	LSKMAGN04
PureProteome™ 0.3 µm Carboxy FlexiBind Magnetic Bead System	2 x 1 mL	LSKMAG03CBX02
	10 mL	LSKMAG03CBX10
PureProteome™ 1.0 µm Carboxy FlexiBind Magnetic Bead System	2 x 1 mL	LSKMAG1CBX02
	10 mL	LSKMAG1CBX10
PureProteome™ 2.5 µm Carboxy FlexiBind Magnetic Bead System	2 x 1 mL	LSKMAG25CBX02
	10 mL	LSKMAG25CBX10
Depletion Kits		
PureProteome™ Human Albumin/Immunoglobulin Depletion Kit	1 kit	LSKMAGHDKIT
PureProteome™ Albumin/IgG Depletion Kit	1 kit	LSKMAGD12
Magnetic Stands		
PureProteome™ Magnetic Stand, 8 tube capacity	1	LSKMAGS08
PureProteome™ Magnetic Stand, 2 x 15 mL tube capacity	1	LSKMAGS15

For more information visit:
www.merckmillipore.com/pureproteome

Site-Specific Proteases and Cleavage Capture Kits

Factor Xa, Thrombin, rEnterokinase, HRV 3C

Remove fusion tags with restriction-grade site-specific proteases (e.g., Thrombin, Biotinylated Thrombin, Factor Xa, HRV 3C protease, Tag•off™ High Activity recombinant enterokinase [rEK], and rEK) and cleavage capture kits. EKapture™ and Xarrest™ agaroses are used for the removal of rEKs and Factor Xa, respectively, following cleavage of fusion proteins. Biotinylated thrombin is removed with streptavidin agarose and HRV 3C is removed with Ni-NTA agarose.

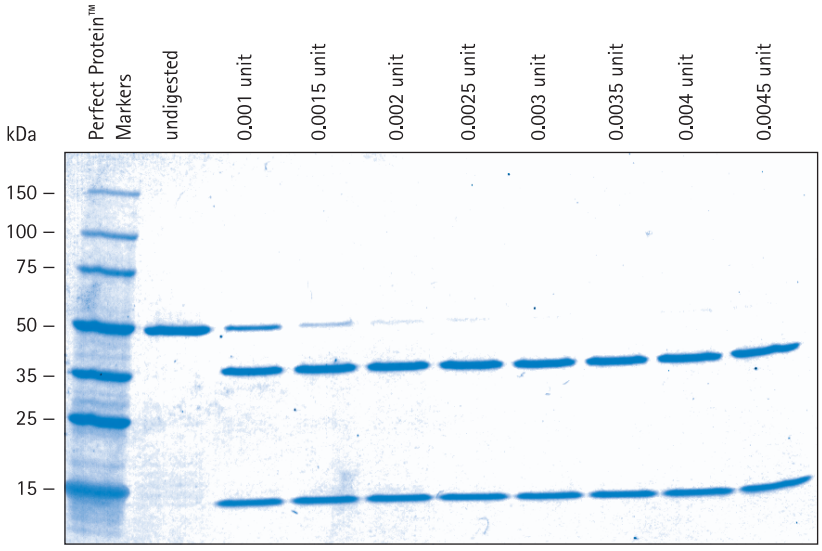
Features & Benefits

- Highly efficient, specific cleavage of fusion proteins
- Recombinant proteases (such as rEK) have higher activity than native protein
- Highly purified
- Functionally tested for activity, free of detectable contaminating proteases
- Supplied with cleavage control protein and buffers
- Capture kits also available

Applications

Removal of Fusion Tags

Product Performance



Biotinylated Thrombin cleavage. The indicated amounts of Biotinylated Thrombin were used to cleave 2 µg of Cleavage Control Protein in an overnight digestion. Samples were analyzed by SDS-PAGE (4–20% gradient gel) followed by staining with Coomassie blue. The 0.0045-unit lane represents a 2.25-fold over-digestion.

Specifications

Description	Components	Unit Definition	Recognition Sequence
Thrombin			
Restriction-Grade Thrombin	50 U Thrombin; 1 mL 10X Thrombin Cleavage Buffer; 2 mL 1X Thrombin Dilution/Storage Buffer; 10 µg Cleavage Control Protein	One unit is defined as the amount of enzyme needed to cleave 1 mg of fusion protein in 16 hours at 20 °C in a 200 µL reaction containing 20 mM Tris-HCl pH 8.4, 150 mM NaCl, 2.5 mM $CaCl_2$, 50 µg fusion protein.	LeuValProArg ↓ GlySer
Biotinylated Thrombin	50 U Biotinylated Thrombin; 1 mL 10X Thrombin Cleavage Buffer; 2 mL 1X Thrombin Dilution/Storage Buffer; 10 µg Cleavage Control Protein		
Thrombin Cleavage Capture Kit	50 U Biotinylated Thrombin; 5 x 1 mL 10X Thrombin Cleavage Buffer; 2 mL 1X Thrombin Dilution/Storage Buffer; 2 x 0.4 mL Streptavidin Agarose; 10 µg Cleavage Control Protein; pkg/10 Spin Filters, 2 mL capacity		

Specifications – Continued

Description	Components	Unit Definition	Recognition Sequence
Restriction Grade Bovine Factor Xa			
Restriction-Grade Factor Xa	100 µg Restriction Grade Factor Xa; 2 mL Factor Xa Dilution/Storage Buffer; 1 mL 10X Factor Xa Cleavage Buffer; 10 µg Xa Cleavage Control Protein	One µg of Restriction Grade Factor Xa cleaves 50 µg Xa Cleavage Control Protein to >95% completion in 16 hours at 25 °C in a buffer containing 50 mM Tris-HCl pH 8.0, 100 mM NaCl, and 5 mM $CaCl_2$.	IleGluGlyArg ↓
Factor Xa Cleavage Capture Kit	100 µg Restriction Grade Factor Xa ; 2 mL Factor Xa Dilution/Storage Buffer ; 5 mL 10X Factor Xa Cleavage/Capture Buffer; 2 × 2.5 mL Xarrest™ Agarose; 10 µg Xa Cleavage Control Protein; pkg/10 Spin Filters, 2 mL capacity		
Recombinant Bovine Enterokinase			
Recombinant Enterokinase	50 U Recombinant Enterokinase; 2 mL 1X rEK Dilution/Storage Buffer; 1 mL 10X rEK Cleavage Buffer; 10 µg Cleavage Control Protein	One unit is defined as the amount of enzyme that will cleave 50 µg of fusion protein in 16 hours at 23 °C, in a buffer containing 20 mM Tris-HCl pH 7.4, 50 mM NaCl, 2 mM $CaCl_2$.	AspAspAspAspLys ↓
Enterokinase Cleavage Capture Kit	50 U Recombinant Enterokinase; 2 mL 1X rEK Dilution/Storage Buffer; 5 mL 10X rEK Cleavage/Capture Buffer; 1.5 mL EKapture™ Agarose ; 10 µg Cleavage Control Protein; pkg/10 Spin Filters, 2 mL capacity		
Tag•off High Activity rEK	50 U Tag•Off™ High-activity rEK; 10 µg Cleavage Control Protein; 2 mL 1X rEK Dilution/Storage Buffer; 1 mL 10X rEK Cleavage Buffer	One unit of Tag•off™ High Activity rEK is defined as the amount of enzyme needed to cleave 50 µg of fusion protein in 16 hours at 23 °C in a buffer containing 20 mM Tris-HCl, 50 mM NaCl, and 2 mM $CaCl_2$, pH 7.4.	AspAspAspAspLys ↓
Tag•off rEK Cleavage Capture Kit	50 U Tag•off™ High Activity rEK; 10 µg Cleavage Control Protein; 2 mL 1X rEK Dilution/Storage Buffer; 5 mL 10X rEK Cleavage/Capture Buffer; 1.5 mL EKapture™ Agarose; 10 Spin Filter, 2 mL		
HRV 3C Protease			
HRV 3C Protease	500 U HRV 3C Protease; 10 µg HRV 3C Cleavage Control Protein; 10 mL 10X HRV 3C Cleavage Buffer	One unit of HRV 3C protease is defined as the amount of enzyme that willl cleave >95% of 100 µg His•Tag® fusion control protein in 50 mM Tris-HCl, 150 mM NaCl, pH 7.5 at 4 °C for 16 h.	LeuGluValLeuPheGln ↓ GlyPro

Ordering Information

Description	Qty/Pk	Catalogue No.
Restriction-Grade Thrombin*	50 U	69671-3
Biotinylated Thrombin*	50 U	69672-3
Thrombin Cleavage Capture Kit	1 kit	69022-3
Cleavage Control Protein	10 µg	69069-3
Restriction-Grade Factor Xa*	100 µg	69036-3
Factor Xa Cleavage Capture Kit	1 kit	69037-3
Xa Cleavage Control Protein	10 µg	69051-3
Xarrast™ Agarose	5 mL	69038-3
Recombinant Enterokinase*	50 U	69066-3
Enterokinase Cleavage Capture Kit	1 kit	69067-3
EKapture™ Agarose	1.5 mL	69068-3
EKapture™ Agarose	10 mL	69068-4
Tag•off™ High Activity rEK*	50 U	71537-3
Tag•off™ rEK Cleavage Capture Kit	1 kit	71540-3
HRV 3C Protease*	500 U	71493-3

*Bulk quantities available. Please inquire.

For more information visit: www.merckmillipore.com/psp

ZipTip® Pipette Tips

Concentrating and purifying samples for MALDI-ToF MS

The ZipTip® pipette tip is a 10 µL tip with a 0.6 or 0.2 µL bed of chromatography media fixed at its end. It is ideal for concentrating and purifying samples for sensitive analyses such as MALDI-ToF MS. The ZipTip® pipette tip provides a reproducible, high-recovery method for concentrating and purifying femtomoles to picomoles of peptides, proteins and oligonucleotides for improved analytical data. To simplify your analysis even further, you can fractionate complex peptide mixtures by step elution.

Features & Benefits

- Single-step desalting, concentration, and purification
- Fractionate complex samples for more meaningful data
- Ideal for peptides, proteins, nucleic acids, and more
- No dead volume for maximum recovery
- Eliminates time-consuming chromatography

Applications

MALDI-ToF MS Sample Preparation

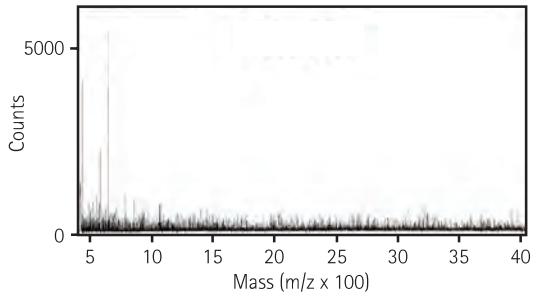
Product Performance

ZipTip® pipette tips increase sensitivity of mass spectrometric analysis. MALDI-ToF MS spectra of a tryptic peptide digest from an in-gel 2D gel digest.

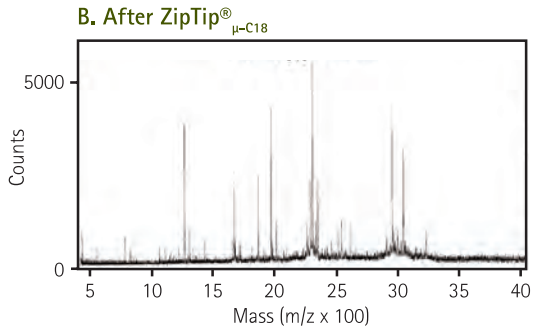
Top spectrum: contaminated sample before clean-up.

Lower spectrum: sample following ZipTip® treatment.

A. Direct Spotting



B. After ZipTip®$_{\mu\text{-C18}}$



Specifications

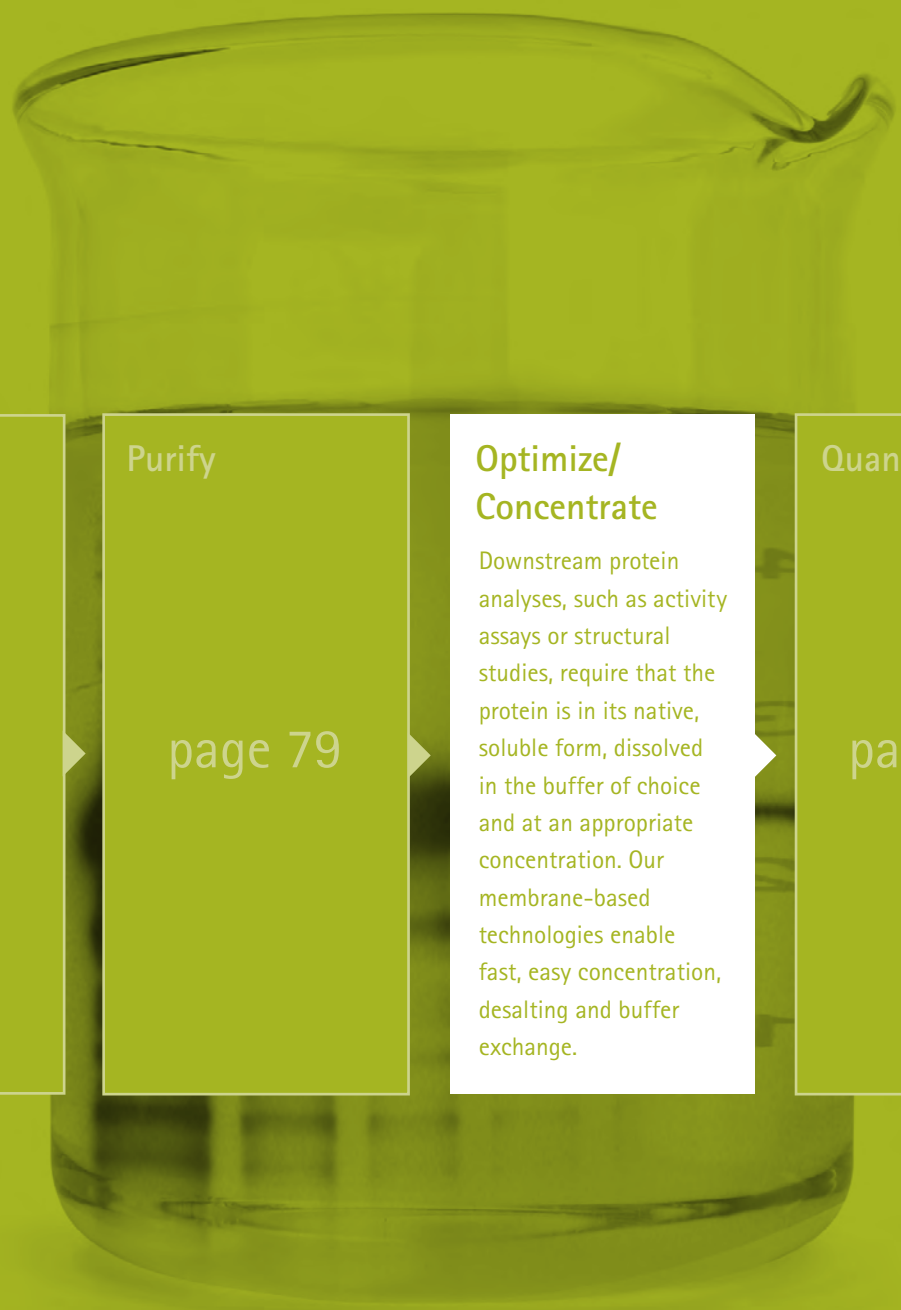
Materials	
Pipette Tip	Polypropylene
C_{18} and µ-C_{18} Resin	Silica, 15 µm, 200 Å pore size
C_4 Resin	Silica, 15 µm, 300 Å pore size
SCX Resin	Strong cation exchange, 12 µm, 300 Å pore size
Volumes, µL	
Pipette Tip	10
Resin Bed	0.2 or 0.6
Capacity	
ZipTip®$_{C18}$ Tip	Typically 5 µg when used with saturating amounts of analyte
ZipTip®$_{\mu\text{-C18}}$ Tip	Typically 2.0 µg when used with saturating amounts of analyte
ZipTip®$_{C4}$ Tip	Typically 3.3 µg when used with saturating amounts of analyte
Dimensions	
Tip Length, mm	31
Tip ID (top), mm	3.375
Tip OD (top), mm	5.8
Temperature, °C	4–70
pH Range	1.5–13.5; 2–12 for 24-hour exposure

Ordering Information

Description	Qty/Pk	Catalogue No.
ZipTip® Pipette Tip, with 0.6 µL C18 resin	8	ZTC18S008
	96	ZTC18S096
	960	ZTC18S960
ZipTip® Pipette Tip, with 0.6 µL C4 resin	8	ZTC04S008
	96	ZTC04S096
	960	ZTC04S960
ZipTip® Pipette Tip, with 0.2 µL C18 resin	8	ZTC18M008
	96	ZTC18M096
	960	ZTC18M960
ZipTip® Pipette Tip, with 0.6 µL strong cation resin (SCX)	8	ZTSCXS008
	96	ZTSCXS096

For more information visit: www.merckmillipore.com/ziptips

Protein Sample Preparation



Extract

page 67

Purify

page 79

Optimize/ Concentrate

Downstream protein analyses, such as activity assays or structural studies, require that the protein is in its native, soluble form, dissolved in the buffer of choice and at an appropriate concentration. Our membrane-based technologies enable fast, easy concentration, desalting and buffer exchange.

Quantify/Detect

page 111

Exchange buffer and concentrate protein solutions over a wide range of sample types and volumes

The limitations of traditional buffer exchange methods include lengthy process times, large volumes of exchange buffer, and risk of sample loss. Merck Millipore's wide selection of sample preparation devices are designed to meets the needs of specific applications. For large sample volumes or proteins that may aggregate if over-concentrated, D-Tube™ dialyzers provide a faster, less tedious alternative to standard dialysis cassettes (Table 1). Amicon® Ultra 4 and 15 mL centrifugal filters are perfectly suited for the concentration of biomarkers from biological fluids with high recovery. The Amicon® Ultra 0.5 mL filter gives the best balance in time and recovery for PCR product cleanup or protein removal prior to analytical HPLC. All Amicon® Ultra filters offer desalting capability with high sample retention. Finally, the Amicon® Pro device enables concentration of a wide range of volumes as well as gentle buffer exchange in a single spin.

Designed for continuous diafiltration (Figure 1), the Amicon® Pro device not only offers speed and efficiency, but also greatly reduces the likelihood of protein aggregation, precipitation or loss of activity. While dialysis is slow and gentle, most spin-based desalting methods involve small devices and multiple rounds of buffer exchange, which can alter protein stability. The Amicon® Pro device's large upper reservoir permits continuous buffer exchange and therefore maintains the sample at constant volume (and concentration), preserving specific activity (Table 1).

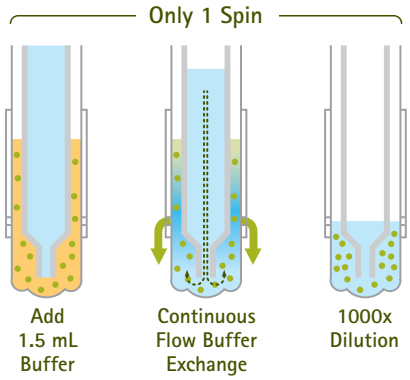


Figure 1. The Amicon® Pro device enables effective desalting or buffer exchange in a single spin, because it features: (1) large, 10 mL buffer reservoir, (2) minimal spacing between the Amicon® Pro device and the attached Amicon® Ultra filter, and (3) tapered exchange tip for optimal buffering. These features ensure that fresh buffer (blue) is slowly but consistently metered in, mixed with sample (green dots in yellow buffer), and expelled through the membrane (green arrows). Because this is a continuous process, the sample does not undergo concentration until buffer exchange is completed.

	Dialysis		Gravity	Centrifugal Diafiltration		
	3 mL Cassette	D-Tube™ Dialyzer	2 mL Column	Device A (0.5 mL)	Amicon® Ultra-0.5 Filter	Amicon® Pro Device
% Salt removal	99.9	99.5	99.6	99.4 (3 spins)	100 (3 spins)	100 (1 spin)
% Protein retention	84.1	87.5	53.3	90.0	92.2	98.4
Process Time	16 Hr	5 Hr	40 min	45-60 min *	45-60 min *	15-30 min *
Specific Activity	4.2	NA	3.4	3.7	3.9	4.3
Concentration required	Yes	Yes	Yes	No	No	No

Table 1. Comparative performance analysis of representative buffer exchange devices. Data represent the results of three independent trials. The Amicon® Pro device provided the most efficient and effective buffer exchange. Dialysis was the most time-consuming, while gravity-driven columns demonstrated significant sample loss, due to fraction pooling and sample transfer. *Process Time depends on whether initial centrifugation was required to concentrate the sample.

For Amicon® Pro System ordering information, see page 80.
For Amicon® Ultra Filter ordering information, see page 98.
For D-Tube™ Dialyzer ordering information, see page 95.

D-Tube™ Dialyzers

High-recovery dialysis of protein and nucleic acids

Easy to handle, D-Tube™ Dialyzers are in a centrifuge tube format with dialysis membrane windows for buffer exchange. Just add or remove your sample with a standard laboratory pipette and obtain >97% sample volume recovery. Available molecular weight cut-offs range from 3.5 to 14 kDa and there are six volume capacities: mini (10–250 µL), midi (50–800 µL), maxi (100– 3000 µL), and mega (10, 15, and 20 mL). The regenerated cellulose membrane is sulfur- and heavy metal-free. Each kit contains 10 D-Tube™ Dialyzers (mega kits also offered in 50/pk) and one floating rack to hold devices in the exchange buffer. Use D-Tube™ Dialyzers for electroelution using the optional accessory kit.

Features & Benefits

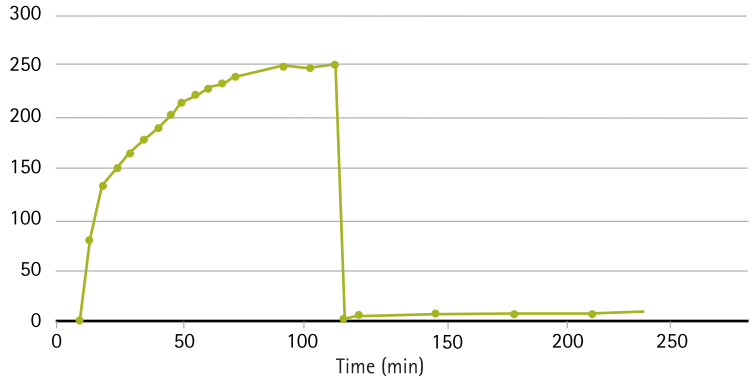
- Ideal for buffer exchange and removal of urea and detergents for samples ranging from 10 µL to 20 mL
- One-step dialysis procedure (no syringes or special equipment)
- Efficient sample recovery
- Protease-, RNase- and DNase-free

Applications

Electroelution of Proteins, Protein-DNA Complexes, DNA, and RNA from Polyacrylamide and Agarose Gels, Dialysis Upstream of MALDI-MS, Functional Assays and HPLC

Product Performance

Experimental Conditions:
Device: D-Tube™ Dialyzer Midi 6,500 MWCO
Sample: BSA 5 mg/mL in 2M NaCl
Volume: 0.5 mL
Exchange buffer volume: 500 mL Milli-Q® water
Conductivity standard curve using NaCl
Protein recovery after 5 hours: 89%
Volume recovery after 5 hours: 115%
Sample conductivity before dialysis: 149 mS/cm/2 M NaCl
Sample conductivity after dialysis: 182 µS/cm/1.6 mM NaCl
99.9% salt reduction



D-Tube™ Dialyzers provide gentle, efficient desalting and excellent protein recovery. A bovine serum albumin (BSA) solution in 2M NaCl was dialyzed against water while water conductivity was monitored. After 100 minutes, conductivity of the water stopped increasing, and it was replaced with fresh water. After 5 hours, 89% of the protein was recovered, and 99.9% of the salt had been removed.

Ordering Information

Description	Volume	MWCO	Qty/Pk	Catalogue No.
Mini	10 - 250 µL	6,000-8,000	10	71504-3
		12,000-14,000	10	71505-3
Midi	50 - 800 µL	3,500	10	71506-3
		6,000-8,000	10	71507-3
Maxi	100 to 3,000 µL	3,500	10	71508-3
		6,000-8,000	10	71509-3
		12,000-14,000	10	71510-3
Mega	10 mL	3,500	10	71739-3
			50	71739-4
		6,000-8,000	10	71740-3
			50	71740-4
Mega	15 mL	3,500	10	71742-3
			50	71742-4
		6,000-8,000	10	71743-3
			50	71743-4

Description	Volume	MWCO	Qty/Pk	Catalogue No.
Mega	20 mL	3,500	10	71745-3
			50	71745-4
		6,000-8,000	10	71746-3
			50	71746-4
D-Tube96™ Dialyzer, 6-8 kDa	10 - 250 µL	6,000-8,000	96 dialyzers	71712-3
D-Tube96™ Dialyzer, 12-14 kDa	10 - 250 µL	12,000-14,000	96 dialyzers	71713-3
D-Tube™ Electroelution Accessory Kit	N/A	N/A	1 kit	71511-3
Mini Floating Racks	N/A	N/A	10	71512-3
Midi Floating Racks	N/A	N/A	10	71513-3
Maxi Floating Racks	N/A	N/A	10	71514-3
Mega Floating Racks	N/A	N/A	10	71748-3

For more information visit: www.merckmillipore.com/psp

Selecting an Ultrafiltration Device

Protein Concentration Devices by Filtration Capacity

Device	Membrane Type	Membrane Orientation	Filtration Capacity 0.5 mL	1 mL	2 mL	4 mL	15 mL	50 mL	70 mL	200 mL	400 mL	Page Number
Small Volume Filtration Devices												
Microcon® Centrifugal Filter	U	H	●									100
Amicon® Ultra-0.5 Centrifugal Filter	U	V	●									98
Amicon® Pro System	U	V	●									80
Ultrafree®-MC Centrifugal Filter	M	H	●									101
Centrifree® Centrifugal Filter	U	H		●								104
Medium Volume Filtration Devices												
Amicon® Ultra-2 Centrifugal Filter	U	V			●							98
Ultrafree®-CL Centrifugal Filter	M	H			●							101
Amicon® Ultra-4 Centrifugal Filter	U	V				●						98
Centriprep® Centrifugal Filter	U	H					●					102
Amicon® Ultra-15 Centrifugal Filter	U	V					●					98
Large Volume Filtration Devices												
8050 Amicon® Stirred Cells	U	H						●				105
Centricon® Plus-70 Centrifugal Device	U	V							●			105
8200 Amicon® Stirred Cells	U	H								●		105
8400 Amicon® Stirred Cells	U	H									●	105

Membrane Type: (U) ultrafiltration vs. (M) microfiltration
Membrane Orientation: (H) horizontal vs. (V) vertical

Selecting an Ultrafiltration Membrane

Application	Ultrafiltration Membrane Nominal Molecular Weight Limit (NMWL) 3,000	10,000	30,000	50,000	100,000
Protein concentration	●	●	●	●	●
Protein purification/desalting/buffer exchange	●	●	●	●	●
Desalting of column fractions	●	●	●	●	●
Protein isolation from cell lysates		●	●		
Peptide concentration/desalting/buffer exchange	●				
Antibody concentration			●	●	●
Virus concentration or removal				●	●
Nucleic acid concentration/desalting/buffer exchange	●	●	●	●	●
Oligonucleotide concentration/desalting/buffer exchange	●				
PCR cleanup				●	●
Remove linkers prior to cloning				●	●
Remove labeled, unincorporated nucleotides			●	●	●
Antibody purification from hybridoma cells			●		
Rapid restriction mapping					●
Natural product screening	●	●	●	●	●
Bound vs. free drugs from serum/plasma (protein removal)		●	●		
Removal of unincorporated label (e.g. fluorescein) from protein	●	●	●	●	●
Removal of imidazole from His-tagged fusion proteins	●	●	●	●	●

Choose the right Amicon® Ultra filter for your molecule of interest

		Amicon® Ultra -0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
Product	Staring Volume	<0.5 mL	<2 mL	<4 mL	<15 mL
	Final Volume	15-20 µL	15-20 µL	50 µL	200 µL
	Concentration Factor	X25-X33	X100-X133	X80	X75
Rotor and G Force	Rotor Adaptor	Standard 1.5 mL	Standard 15 mL	Standard 15 mL	Standard 50 mL
	Fixed-Angle (35°) Rotor	14,000 g 1,000 g reverse spin	7,500 g 1,000 g reverse spin	5,000 g for 100 kDa 7,500 g for all other MWCO	5,000 g
	Swinging Bucket Rotor	N/A	4,000 g 1,000 g reverse spin	4,000 g	4,000 g

	Size	MWCO	Amicon® Ultra -0.5	Amicon® Ultra-2	Amicon® Ultra-4	Amicon® Ultra-15
Applications	**Protein**					
	6 < MW < 20k	3,000	30 min	60 min	40 min	40 min
	20 < MW < 60k	10,000	15 min	40 min	15 min	20 min
	60 < MW < 100k	30,000	10 min	20 min	10 min	20 min
	100 < MW < 200k	50,000	10 min	15 min	10 min	15 min
	200 < MW	100,000	10 min	30 min	10 min	15 min
	Nucleic Acid (single- & double-stranded)					
	137-1159 bp	30,000	10 min	20 min	10 min	20 min
	Nanoparticles					
	1.5 < dia < 3 nm	3,000	30 min	60 min	40 min	40 min
	3 < dia < 5 nm	10,000	15 min	40 min	15 min	20 min
	5 < dia < 7 nm	30,000	10 min	20 min	10 min	20 min
	7 < dia < 10 nm	50,000	10 min	15 min	10 min	15 min
	10 nm < dia	100,000	10 min	30 min	10 min	15 min

Amicon® Ultra Centrifugal Filters

Fast and easy sample concentration

- Broad chemical compatibility: Heat-sealed membrane eliminates adhesives and downstream extractables; compatible with pH 1 to 9
- Reverse spin recovery: Enables maximum protein recovery without introducing pipetting errors; low binding membrane and polypropylene housing for > 90% sample recovery
- Reliable: Spin precious samples with confidence in one robust, sleek unit that prevents leakage

Applications

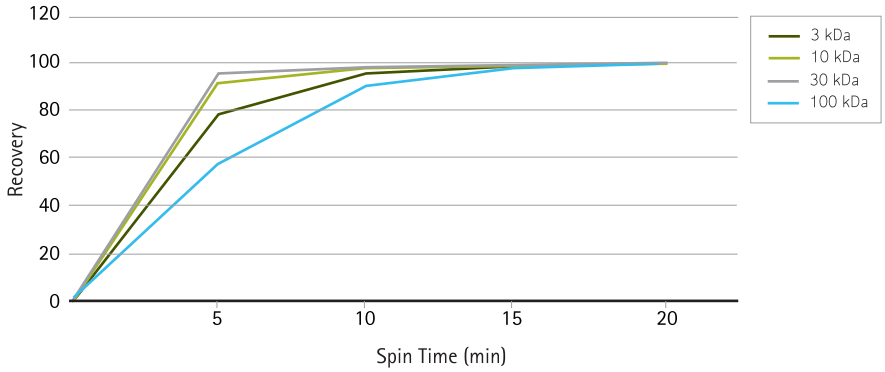
Protein Concentration, Nucleic Acid Concentraion, Buffer Exchange, Dialysis, Desalting

Amicon® Ultra centrifugal filters provide fast sample processing and promote high sample recoveries, even in dilute samples, through ultrafiltration. The unique features of the Amicon® Ultra centrifugal filters give you the fastest, most efficient concentration for sensitive downstream applications.

Features & Benefits

- Dead stop: Avoids spinning to dryness, provides a predictable concentration factor and no need to calibrate for several samples to run in parallel
- Vertical membranes: Aligned with filtrate rather than perpendicular for less clogging, less waste and faster filtration; capable of 25- to 80-fold concentration in a single step

Product Performance



Amicon® Ultra 4 mL filters provide fast spin times with excellent recovery. Solutions of four different proteins (3 kDa cytochrome C, 10 kDa cytochrome C, 30 kDa BSA and 100 kDa IgG) were concentrated using Amicon® Ultra 4 mL filters. Average percent recovery and spin times were recorded. Data show that more than 95% of all protein was recovered in 15 minutes or less.

Ordering Information

Description	Maximum Initial Sample Volume (mL)	Final Concentrate (Retentate) Volume (µL)	MWCO	Qty/Pk	Catalogue No.
Amicon® Ultra-0.5 Centrifugal Filters	0.5	15–20	3,000	8	UFC500308
				24	UFC500324
				96	UFC500396
				500	UFC5003BK
			10,000	8	UFC501008
				24	UFC501024
				96	UFC501096
				500	UFC5010BK
			30,000	8	UFC503008
				24	UFC503024
				96	UFC503096
				500	UFC5030BK
			50,000	8	UFC505008
				24	UFC505024
				96	UFC505096
				500	UFC5050BK
			100,000	8	UFC510008
				24	UFC510024
				96	UFC510096
				500	UFC5100BK

Ordering Information – Continued

Description	Maximum Initial Sample Volume (mL)	Final Concentrate (Retentate) Volume (µL)	MWCO	Qty/Pk	Catalogue No.
Amicon® Ultra-2 Centrifugal Filters	2	15–20	3,000	24	UFC200324
			10,000	24	UFC201024
			30,000	24	UFC203024
			50,000	24	UFC205024
			100,000	24	UFC210024
Amicon® Ultra-4 Centrifugal Filters	4	30–70	3,000	8	UFC800308
				24	UFC800324
				96	UFC800396
			10,000	8	UFC801008*
				24	UFC801024*
				96	UFC801096*
			30,000	8	UFC803008
				24	UFC803024
				96	UFC803096
			50,000	8	UFC805008
				24	UFC805024
				96	UFC805096
			100,000	8	UFC810008
				24	UFC810024
				96	UFC810096
Amicon® Ultra-15 Centrifugal Filters	15	150–300	3,000	8	UFC900308
				24	UFC900324
				96	UFC900396
			10,000	8	UFC901008*
				24	UFC901024*
				96	UFC901096*
			30,000	8	UFC903008
				24	UFC903024
				96	UFC903096
			50,000	8	UFC905008
				24	UFC905024
				96	UFC905096
			100,000	8	UFC910008
				24	UFC910024
				96	UFC910096

*Certified for clinical applications.

For more information visit: **www.merckmillipore.com/amicon**

Microcon® Centrifugal Filters

For DNA and protein concentration

Simply and efficiently concentrate and desalt solutions of any macromolecule, using any centrifuge that can accept 1.5 mL tubes. With the low-binding Ultracel® membrane, Microcon® filters offer typical recoveries of >95%, even for dilute solutions; reverse spin to maximize recovery, even in the smallest samples; convenient storage of filtrate or concentrated sample in standard microfuge tubes and concentration factors up to 100X.

Applications

Recovery of Genomic DNA for Forensic Applications, Concentration and Desalting of Nucleic Acids (high recovery alternative to ethanol precipitation), Removal of Primers from Amplified DNA, Protein Concentration

Features & Benefits

- High, easy recovery for small volumes with reverse spin (concentration factor <20X)
- Low-binding Ultracel® membrane
- Fast processing

Specifications

Volumes	
Maximum Initial Sample Volume	500 µL
Typical Final Concentration Volume	5 -50 µL
Active Membrane Area	0.32 cm^2
Hold-up Volume	≤10 µL
Optimal and Maximum Relative Centrifugal Force – Fixed Angle Rotor	Microcon® 10K devices - 14,000 x g Microcon® 30K devices - 14,000 x g Microcon® DNA Fast Flow - 500 x g 1,000 x g for recovery spin
Materials	
Filter Device	Polycarbonate
Membrane	Ultracel® low-binding regenerated cellulose
Collection Tube	Polypropylene
O-Ring	Medical-grade silicone rubber
Dimensions	
Diameter	12.3 mm (0.5 in.)
Length (filter device and tube in concentration mode)	45.0 mm (1.8 in.)
Length (filter device and tube in recovery mode)	48.2 mm (1.9 in.)

Ordering Information

Description	Volume (mL)	Min. final concentrate volume (µL)	Qty/Pk	Catalogue No.
Microcon® Filter, Ultracel® membrane, 10 kDa	0.5	5-50	100	MRCPRT010
Microcon® Filter, Ultracel® membrane, 30 kDa	0.5	5-50	100	MRCF0R030
Microcon® Filter, Ultracel® DNA Fast Flow Membrane	0.5	5-50	100	MRCF0R100
Microcon® Filter, Ultracel® DNA Fast Flow PCR Grade Membrane	0.5	5-50	20	MRCF0R100ET

For more information visit: www.merckmillipore.com/psp

Ultrafree®-MC and -CL Centrifugal Filter Units

Fast and easy microfiltration

Ultrafree®-MC (0.5 mL) and Ultrafree®-CL (2 mL) centrifugal devices are single-use, disposable filters used for removing particulates from aqueous biological solutions. These devices are available in two processing volumes with a range of microporous membranes (from 0.1 to 5.0 µm) for fast filtration and highly reproducible performance. Pre-sterilized units are also available. Use in fixed-angle rotors for 1.5 mL tubes (-MC) and 15 mL tubes (-CL).

Features & Benefits

- Five different pore sizes from 0.1 to 5.0 µm
- Available in Durapore® PVDF or hydrophilic PTFE membranes
- Pre-sterilized units also available
- Fast filtration and highly reproducible performance

Specifications

	Ultrafree®-MC Filter	Ultrafree®-CL Filter
Volumes		
Maximum Initial Sample Volume, mL	0.5	2
Hold-up Volume, µL	5	10
Centrifugal Force	12,000 x g	5,000 x g
Dimensions		
Active Membrane Area, cm^2	0.2	0.8
Diameter, mm	10.6	16.3
Length, mm	45	77
Materials		
Membrane	Hydrophilic PVDF or hydrophilic PTFE	Hydrophilic PVDF or hydrophilic PTFE
Device	Polypropylene	Polypropylene

Applications

Clarification of Aqueous and Some Solvent-based Samples, Protein and Nucleic Acid Sample Preparation

Ordering Information

Description	Pore Size (µm)	Color	Sterility	Qty/Pk	Catalogue No.
Filter Units with Microporous Durapore® PVDF Membrane					
Ultrafree®-MC Filter	0.1	Orange	Non-Sterile	25	UFC30VV25
				100	UFC30VV00
	0.22	Yellow	Non-Sterile	25	UFC30GV25
				100	UFC30GV00
				250	UFC30GVNB
			Sterile	50 (5 x 10)	UFC30GV0S
	0.45	Red	Non-Sterile	25	UFC30HV25
				100	UFC30HV00
				250	UFC30HVNB
	0.65	Purple	Non-Sterile	25	UFC30DV25
				100	UFC30DV00
			Sterile	50 (5 x 10)	UFC30DV0S
	5	Dark green	Non-Sterile	100	UFC30SV00
Ultrafree®-CL Filter	0.1	Orange	Non-Sterile	25	UFC40W25
				100	UFC40W00
	0.22	Yellow	Non-Sterile	25	UFC40GV25
				100	UFC40GV00
			Sterile	50 (5 x 10)	UFC40GV0S
	0.45	Red	Non-Sterile	25	UFC40HV25
				100	UFC40HV00
	0.65	Purple	Non-Sterile	25	UFC40DV25
	5	Dark Green	Non-Sterile	25	UFC40SV25

Ordering Information – Continued

Description	Pore Size (µm)	Color	Sterility	Qty/Pk	Catalogue No.
Filter Units with Microporous Hydrophilic PTFE Membrane					
Ultrafree®-MC Filter	0.22	Yellow	Non-Sterile	25	UFC30LG25
	0.45	Red	Non-Sterile	25	UFC30LH25
Ultrafree®-CL Filter	0.22	Yellow	Non-Sterile	25	UFC40LG25
	0.45	Red	Non-Sterile	25	UFC40LH25

For more information visit: www.merckmillipore.com/psp

Centriprep® Centrifugal Filters

Concentration of samples with high solute content

Centriprep® centrifugal filters are disposable ultrafiltration devices used for purifying, concentrating, and desalting biological samples in the 2–15 mL volume range. They may also be used for filtration applications. These complete, ready-to-use ultrafiltration devices are designed for operation in most centrifuges that can accommodate 50 mL centrifuge tubes. They are easy to use and offer a high flow rate. Centriprep® devices consist of a sample container with a twist-lock cap, a filtrate collector containing a low-adsorptive, Ultracel® regenerated cellulose membrane and an air-seal cap for sample isolation.

Features & Benefits

- For use with samples containing high levels of solutes
- Unique inverse flow mode of operation with large deadstop
- Fast sample processing
- Available with Ultracel® membranes: (3, 10, 30 and 50 kDa NMWL)
- Fits standard swinging-bucket rotor for 50 mL tubes

Applications

Concentration and Purification of Particle-laden Solutions, Solutions with High Solute Concentration, Separation of Low MW Solutes from Fermentation Broths, Cell Culture Media and Lysates

Ordering Information

Description	Volume (mL)	Min. Final Concentrate Volume (µL)	MWCO	Qty/Pk	Catalogue No.
Centriprep® Filter With Ultracel® Membrane, 3 kDa NMWL	15	700	3,000	24	4302
				96	4303
Centriprep® Filter With Ultracel® Membrane, 10 kDa NMWL*	15	700	10,000	24	4304
				96	4305
Centriprep® Filter With Ultracel® Membrane, 30 kDa NMWL*	15	700	30,000	24	4306
				96	4307
Centriprep® Filter With Ultracel® Membrane, 50 kDa NMWL	15	700	50,000	24	4310
				96	4311

*Centriprep® centrifugal filter devices with Ultracel® 10 kDa and 30 kDa membranes are approved for *in vitro* diagnostic use.

For more information visit: www.merckmillipore.com/psp

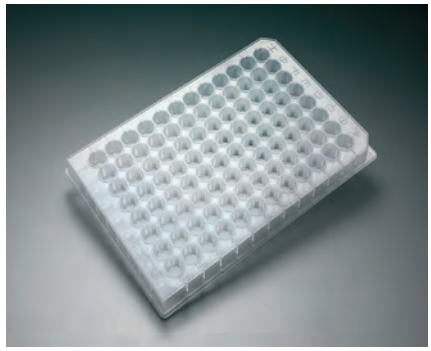
MultiScreen® Filter Plate with Ultracel®-10 Membrane

High-throughput ultrafiltration

The MultiScreen® ultrafiltration-based filter plate enables high-throughput, automation-compatible sample purification, concentration and desalting of biological solutions and protein removal from samples prior to analysis. The 96-well filter plate incorporates Ultracel® 10,000 nominal molecular weight limit regenerated cellulose ultrafiltration membrane for low-binding, high-recovery results. It is designed for use with centrifugation and is compatible with standard microtiter plate instrumentation and liquid handling equipment.

Features & Benefits

- High throughput: 96-well ultrafiltration plate
- Reliable: Designed for low protein binding, high protein retention and high well-to-well uniformity of performance
- Versatile: Allows for processing and collection of sample volumes from 50 to 500 µL and is compatible with a range of standard receiver 96-well microtiter plates



Applications

High-Throughput Protein Concentration and Desalting for Validation or Screening Assays, Removal of Large Molecular Weight Proteins Prior to Instrument Analysis

Specifications

Volumes	
Filter Plate Capacity, mL	0.5
Working Sample Volume Capacity	Limited to receiver plate. Compatible with standard 96-well microtiter plates (300 µL, 700 µL deep well, and 150 µL conical u-bottom).*
Centrifugal Force	Maximum 3,000 x g
Materials	
Membrane	Ultracel® regenerated cellulose
Device	Polyolefin
Dimensions	
Length, mm	128
Width, mm	85.2
Height, mm	14.2

*Recommended receiver plates sold separately.

Ordering Information

Description	Qty/Pk	Catalogue No.
MultiScreen® Filter Plate with Ultracel® Membrane, 10,000 NMWL	10	MAUF01010

Includes MultiScreen® filter plates and lids. Receiver plate must be purchased separately.

For more information visit: **www.merckmillipore.com/psp**

Centrifree® Ultrafiltration Device

Separate free from unbound solutes

The Centrifree® Ultrafiltration device with Ultracel® regenerated cellulose membrane is ideal for separating free from bound microsolutes in serum, plasma, and other biological samples. The device is licensed for research use and *in vitro* diagnostic use. The device holds a sample volume of 1 mL with a hold-up volume of 10 µL.

Features & Benefits

- Separate unbound (free) therapeutic drugs, testosterone, thyroxin, etc.
- Enables accuracy in binding studies
- Critical for new drug investigations
- Facilitates deproteinization to reduce complexity or matrix interference of biological samples
- For research and *in vitro* diagnostic use

Applications

Determination of Free Drugs, Testosterone, Thyroxin or Other Bioactives, Binding Studies, New Drug Investigations

Ordering Information

Description	Volume (mL)	Min. Final Concentrate Volume (µL)	Qty/Pk	Catalogue No.
Centrifree® Ultrafiltration Device with Ultracel® Membrane	1	50	50	4104

For more information visit: www.merckmillipore.com/psp

Minicon® Concentrators

Static concentration of bodily fluids

Use the Minicon® static concentrators to concentrate urine and cerebrospinal fluid to intensify proteins that indicate abnormal or pathological states prior to analysis by electrophoresis or immunoelectrophoresis. The B15 unit has 8 cells and can hold up to 5 mL of sample. The CS15 has 10 cells and is suited for volumes up to 2.5 mL. The absorbent pulls solvent and salts through the ultrafilter, concentrating the sample.

Features & Benefits

- Static concentrator, requiring no accessories
- Absorbent pulls solvent and salts through ultrafilter, concentrating sample
- Concentration factor up to 100X
- Low hold-up volume

Applications

Concentration of Urine and Cerebrospinal Fluid for Enrichment of Protein Analytes (e.g., Bence Jones Proteins in Urine)

Ordering Information

Description	MWCO	Volume (mL)	Min. Final Concentrate Volume (µL)	Qty/Pk	Catalogue No.
Minicon® B15 Concentrator, 8 Cells/Unit	15,000	5	50	40	9031
Minicon® CS15 Concentrator, 10 Cells/Unit	15,000	2.5	30	50	9051

For more information visit: www.merckmillipore.com/psp

Centricon® Plus-70 Centrifugal Filter Units

Large volume sample concentration

Concentrate samples in the 15 mL-70 mL volume range using Centricon® Plus-70 centrifugal filter units. The large deadstop volume of 350 µL prevents spinning samples to dryness. The Centricon® Plus-70 has two vertical regenerated cellulose membranes for fast, efficient tangential flow. The device is available with four different pore size membranes (3, 10, 30 and 100 kDa NMWL).

Features & Benefits

- 90% typical recovery
- 50X to 200X concentration
- Low hold-up volume
- Deadstop prevents spinning to dryness
- Polypropylene housing minimizes non-specific protein binding

Applications

Concentration and Desalting of Chromatography Column Eluates, Concentrating Monoclonal Antibodies, Concentrating Proteins or Viruses From Culture Supernatants, Clarifying Tissue Homogenates and Lysates, Buffer Exchange or Diafiltration

Ordering Information

Description	MWCO	Qty/Pk	Catalogue No.
Centricon® Plus-70 Filter With Ultracel® Membrane	3,000	8	UFC700308
	10,000	8	UFC701008
	30,000	8	UFC703008
	100,000	8	UFC710008

For more information visit: www.merckmillipore.com/psp

Stirred Cells

Pressure-based sample concentration

Need to concentrate your sample gently, without centrifugation? Amicon® stirred cells concentrate samples rapidly but gently, using magnetic stirring to minimize concentration polarization and shear stress-induced denaturation. Providing high flow rates with solutions up to 10% macrosolute concentration, Amicon® stirred cells also enable salt removal followed by concentration in the same unit. Complete product recoveries can generally be achieved using the diafiltration set-up. All stirred cells can be autoclaved. Amicon® stirred cells are available in five different sizes: 3 mL, 10 mL, 50 mL, 200 mL and 400 mL.

Features & Benefits

- Rapid concentration
- Available in five different sizes
- Simple, easy-to-use system
- Autoclavable
- Open platform is compatible with different membranes

Applications

Concentration, Buffer Exchange, Desalting

Specifications

Materials	
Cap and Tube Fitting Assembly	Nylon
Cylinder and Membrane Holder	Polysulfone
Stirring Assembly	Acetal, polysulfone
O-rings	Silicone rubber
Pressure Tube	Polyethylene
Filtrate Tube	Tygon® tubing
Retaining Stand	Anodized aluminum or nylon

Amicon® Stirred Cell	Model 8003 Cat. No. 5125	Model 8010 Cat. No. 5121	Model 8050 Cat. No. 5122	Model 8200 Cat. No. 5123	Model 8400 Cat. No. 5124
Max. Process Volume, mL	3	10	50	200	400
Min. Process Volume, mL	0.075	1.0	2.5	5.0	10.0
Membrane Diameter, mm	25	25	44.5	63.5	76
Effective Membrane Area, cm^2	0.9	4.1	13.4	28.7	41.8
Hold-up Volume, mL	0.07	0.2	0.5	1.2	1.5
Height, cm	7.7	7.7	9.8	12.8	15.5
Base, cm	6 x 6	6 x 6	7 x 7	9 x 9	11 x 11
Weight, kg	0.1	0.1	0.2	0.4	0.6
Maximum Operating Pressure, psi	75	75	75	75	75

Ordering Information

Description	Min. Volume (mL)	Max. Volume (mL)	Qty/Pk	Catalogue No.
Amicon® Stirred Cells	0.075	3	1	5125
	1.0	10	1	5121
	2.5	50	1	5122
	5.0	200	1	5123
	10	400	1	5124
Replacement Parts				
Small Replacement Parts for 8000 Series			1	8000SCPKIT
Large Replacement Parts for 8003 Series			1	8003SCPKIT
Large Replacement Parts for 8010 Series			1	8010SCPKIT
Large Replacement Parts for 8050 Series			1	8050SCPKIT
Large Replacement Parts for 8200 Series			1	8200SCPKIT
Large Replacement Parts for 8400 Series			1	8400SCPKIT
Accessories				
CDS10 Selector Valve			1	6003
MF2 Push-Button Manifold			1	6015
RC800 Mini-Reservoir			1	6028

For more information visit: www.merckmillipore.com/psp

Solvent-Resistant Stirred Cells

Concentration of samples in organic solvents

For concentration or buffer exchange of samples containing organic solvents, this stirred cell is available in two sizes, a 47 mm cell and a 76 mm cell. However, certain aldehydes, ketones (e.g., acetone) and aliphatic ethers/esters may reduce the life of the fluorocarbon O-rings.

Features & Benefits

- Borosilicate glass cylinder and PTFE components for broad compatibility
- Autoclavable with membrane in place
- Top plate opening provides access to contents without dismantling
- Few components, easy to clean and assemble
- For 47 or 76 mm disc filters

Applications

Concentration and Buffer Exchange Involving Organic Solvents

Specifications

Materials		
Top and base plate	316L stainless steel	
Cylinder	Borosilicate glass	
Stirring bar grip and coating	PTFE	
O-ring	Fluorocarbon	
Pressure tube	Nylon	
Filtrate tube	Silicone rubber	
Funnel	HDPE	
Volumes	**47 mm cell**	**76 mm cell**
Max. Process Volume, mL	75	300
Min. Process Volume, mL	2.5	10
Hold-up Volume, mL	0.3	1
Dimensions		
Filter Diameter, mm	47	76
Filtration Area, cm^2	15	40
Base Diameter, cm	8	11
Height, cm	12.2	16.5
Maximum Operating Pressure, bar (psi)	6.2 (90)	6.2 (90)

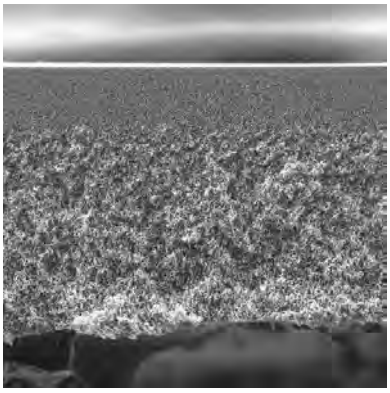
Ordering Information

Description	Minimum Volume (mL)	Maximum Volume (mL)	Qty/Pk	Catalogue No.
Solvent-resistant Stirred Cell, for 47 mm membranes	2.5	75	1	XFUF04701
Solvent-resistant Stirred Cell for, 76 mm membranes	10	300	1	XFUF07601
Replacement Parts				
Glass Cylinder for 47 mm Stirred Cell			1	XFUF04711
Glass Cylinder for 76 mm Stirred Cell			1	XFUF07611

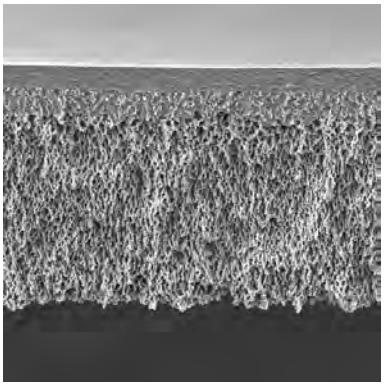
For more information visit: www.merckmillipore.com/psp

Ultrafiltration Discs

Membranes for use with stirred cells



Biomax® ultrafiltration membrane



Ultracel® ultrafiltration membrane

Assemble your stirred cell with the membrane of your choice. Amicon® Ultrafiltration cut disc membranes are available in regenerated cellulose and Biomax® polyethersulfone (PES). The membranes are available in a range of different pore sizes and diameters. Ultracel® regenerated cellulose membranes are recommended for concentrating or desalting dilute solutions. The hydrophilic, tight microstructure of Ultracel® membranes assures the highest possible retention with the lowest possible adsorption of protein, DNA or other macromolecules. Biomax® PES membranes are recommended for concentrating or desalting higher volumes of more concentrated samples such as serum, plasma, or conditioned tissue culture media.

Features & Benefits

- Disc diameters include: 25, 44.5, 47, 63.5, 76, 90 and 150 mm
- Both Ultracel® and Biomax® membranes are available in a wide range of pore sizes to meet your separation requirements
- Compatible with Amicon® Stirred Cells

Applications

Concentration, Buffer Exchange, Desalting

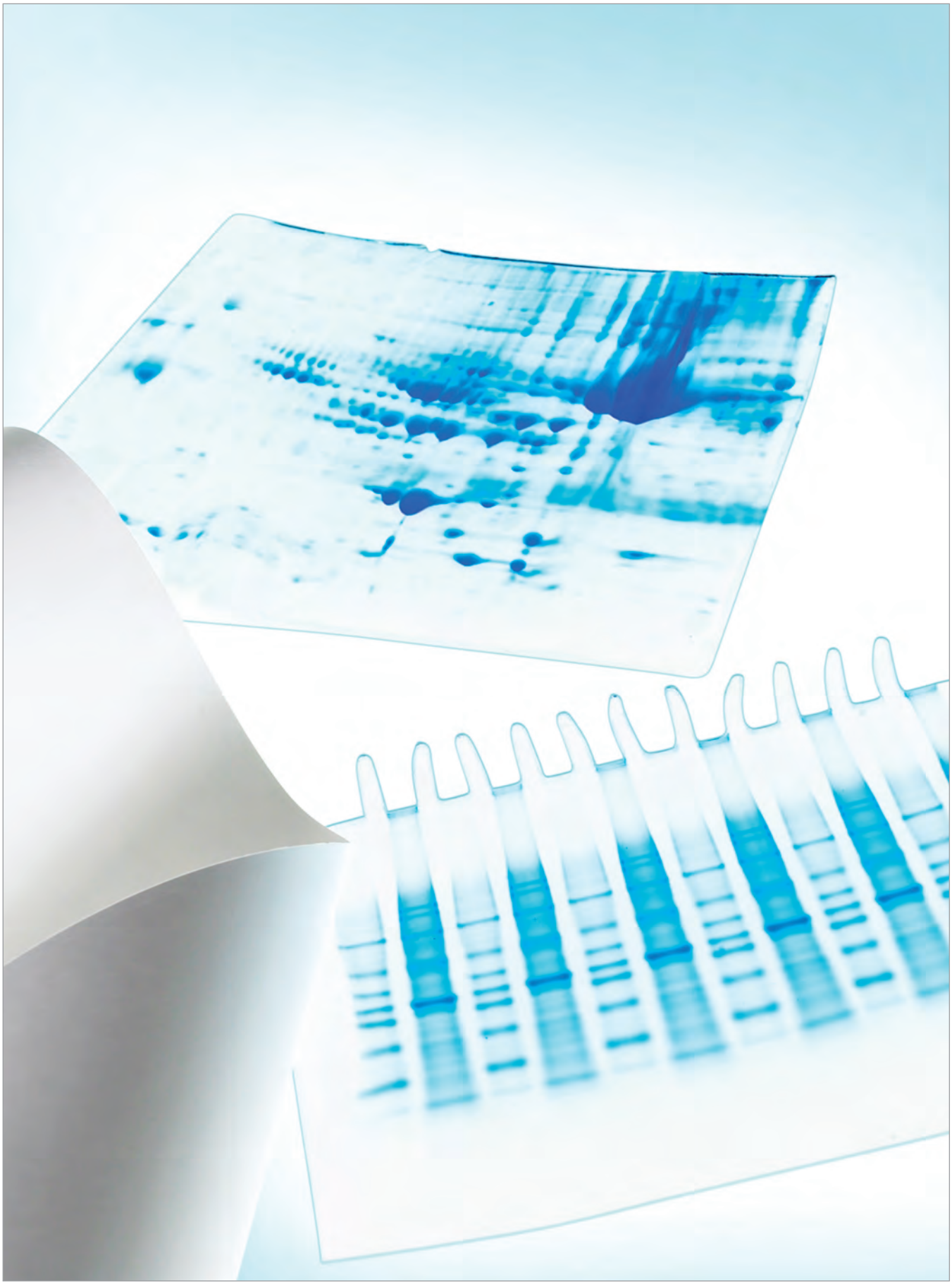
Ordering Information – Ultracel® Ultrafiltration Discs (Regenerated Cellulose)

Description	Filter Diameter (mm)	Qty/Pk	Catalogue No.
Ultracel® Membrane, 1,000 MWCO	25	10	PLAC02510
	44.5	10	PLAC04310
	47	10	PLAC04710
	63.5	10	PLAC06210
	76	10	PLAC07610
	90	5	PLAC09005
	150	5	PLAC15005
Ultracel® Membrane, 3,000 MWCO	25	10	PLBC02510
	44.5	10	PLBC04310
	47	10	PLBC04710
	63.5	10	PLBC06210
	76	10	PLBC07610
	90	5	PLBC09005
	150	5	PLBC15005
Ultracel® Membrane, 5,000 MWCO	25	10	PLCC02510
	44.5	10	PLCC04310
	47	10	PLCC04710
	63.5	10	PLCC06210
	76	10	PLCC07610
	90	5	PLCC09005
	150	5	PLCC15005
Ultracel® Membrane, 10,000 MWCO	25	10	PLGC02510
	44.5	10	PLGC04310
	47	10	PLGC04710
	63.5	10	PLGC06210
	76	10	PLGC07610
	90	5	PLGC09005
	150	5	PLGC15005
Ultracel® Membrane, 30,000 MWCO	25	10	PLTK02510
	44.5	10	PLTK04310
	47	10	PLTK04710
	63.5	10	PLTK06210
	76	10	PLTK07610
	90	5	PLTK09005
	150	5	PLTK15005
Ultracel® Membrane, 100,000 MWCO	25	10	PLHK02510
	44.5	10	PLHK04310
	47	10	PLHK04710
	63.5	10	PLHK06210
	76	10	PLHK07610
	90	5	PLHK09005
	150	5	PLHK15005

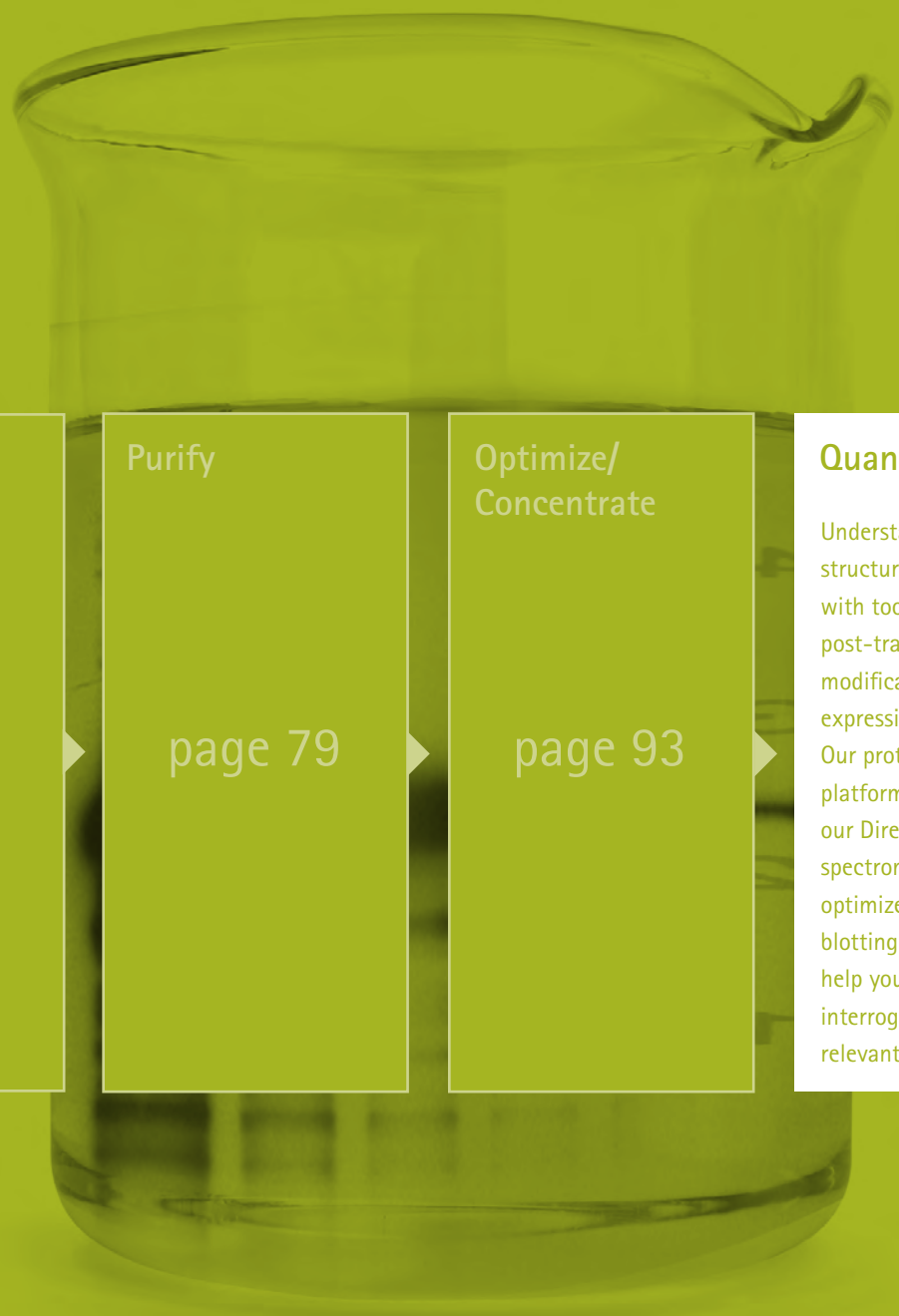
Ordering Information – Biomax® Ultrafiltration Discs (Polyethersulfone)

Description	Filter Diameter (mm)	Qty/Pk	Catalogue No.
Biomax® Membrane, 5,000 MWCO	25	10	PBCC02510
	44.5	10	PBCC04310
	47	10	PBCC04710
	63.5	10	PBCC06210
	76	10	PBCC07610
Biomax® Membrane, 10,000 MWCO	25	10	PBGC02510
	44.5	10	PBGC04310
	47	10	PBGC04710
	63.5	10	PBGC06210
	76	10	PBGC07610
Biomax® Membrane, 30,000 MWCO	25	10	PBTK02510
	44.5	10	PBTK04310
	47	10	PBTK04710
	63.5	10	PBTK06210
	76	10	PBTK07610
Biomax® Membrane, 50,000 MWCO	25	10	PBQK02510
	44.5	10	PBQK04310
	47	10	PBQK04710
	63.5	10	PBQK06210
	76	10	PBQK07610
Biomax® Membrane, 100,000 MWCO	25	10	PBHK02510
	44.5	10	PBHK04310
	47	10	PBHK04710
	63.5	10	PBHK06210
	76	10	PBHK07610
Biomax® Membrane, 300,000 MWCO	25	10	PBMK02510
	44.5	10	PBMK04310
	47	10	PBMK04710
	63.5	10	PBMK06210
	76	10	PBMK07610
Biomax® Membrane, 500,000 MWCO	25	10	PBVK02510
	44.5	10	PBVK04310
	47	10	PBVK04710
	63.5	10	PBVK06210
	76	10	PBVK07610

For more information visit: **www.merckmillipore.com/psp**



Protein Sample Preparation



Extract

page 67

Purify

page 79

Optimize/ Concentrate

page 93

Quantify/Detect

Understand protein structure and function with tools to characterize post-translational modifications and expression control. Our protein detection platforms, including our Direct Detect® spectrometer and optimized Western blotting products, help you accurately interrogate the proteins relevant to your system.

Simplified analysis of lipid or detergent content in biological samples using the IR-based Direct Detect® Spectrometer

Despite the growth in lipid research, analytical methods applied for their characterization typically involve multistep procedures, requiring extensive sample manipulation and separation from other biomolecules, like proteins, before the analysis.

The infrared (IR)-based Direct Detect® spectrometer enables simultaneous protein quantitation and lipid analysis, directly from a biological sample. Given that each lipid possesses an IR signature uniquely defined by its chemical composition and structure, IR spectroscopy offers a means of qualitative lipid discrimination, as well as quantitation of known lipid(s) in cases where a viable standard curve has been determined.

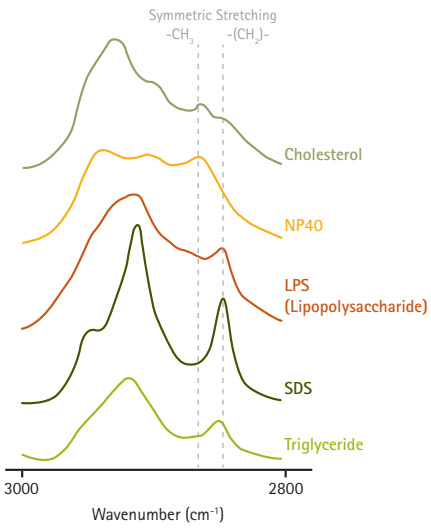


Figure 1. C-H symmetric stretching bands observed in the infrared spectra of lipids.

The Direct Detect® spectrometer utilizes the C-H symmetric stretching vibrational population between 2870 and 2840 cm^{-1} to determine lipid or detergent content (Figure 1). The Direct Detect® assay-free sample card enables analysis of aqueous-based biological samples, which are normally not compatible with infrared spectroscopy, due to their high water content. These assay-free cards are also compatible with many organic solvents.

Materials and Methods

Measurements of sample concentration were acquired using Direct Detect® assay-free sample cards (Merck Millipore, Cat. No. DDAC00010-8P) and the Direct Detect® spectrometer (Merck Millipore, Cat. No. DDHW00010-WW). Each assay-free card contains four polytetrafluoroethylene (PTFE) membrane positions, sized for easy sample application and analysis. All measurements were performed using 2 µL of sample solution per membrane position. Unknown lipid mixtures were analyzed in the "Relative Absorbance" mode, where the system delivers information based solely on IR signal strength. Empirical sample concentration values were determined by interpolation from calibration curves developed for each specific lipid or detergent. For the experiments reported here, the system was calibrated using Tetracosanoic Acid in chloroform and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) in phosphate-buffered saline (PBS). A series of seven concentrations from 0.25 mg/mL to 1.75 mg/mL was used to generate an instrument calibration curve for Tetracosanoic Acid. For CHAPS, the calibration curve was formed using a series of seven concentration points spanning 0.25% to 4%. The strength of IR signal for each concentration was fitted to a regression line represented by linear equations $y = 0.04519x - 0.00679$ (lipid) and $y = 0.01774x + 0.00442$ (detergent). These equations were used by the Direct Detect® software to determine the concentration of Tetracosanoic Acid and CHAPS in subsequent samples.

Robustness of the Relative Absorbance mode was further demonstrated through simultaneous analysis of protein and lipid content in samples containing mixture of both biomolecules as well as in breast cancer tissue lysate. Frozen tissue, derived from a breast ductal carcinoma, was divided into 2 equal samples. Tissue was covered with 2 mL RIPA buffer (Merck Millipore, Cat. No. 20-188) or CytoBuster™ protein extraction reagent (Merck Millipore, Cat. No. 71009-50mL), both supplemented with an inhibitor cocktail, and disrupted with a glass tissue homogenizer. Effective removal of the fatty fraction from the resulting tissue homogenate by a series of centrifugation steps was also monitored by the Direct Detect® spectrometer.

Results

Accuracy of lipid and detergent quantitation

The accuracy of concentration estimation within the dynamic ranges established for Tetracosanoic Acid and CHAPS was assessed using 0.8 mg/mL Tetracosanoic Acid in chloroform and 1.8 % CHAPS in PBS. Within a well-defined calibration method, the instrument was capable of estimating lipid and detergent concentration with minimal error: the Direct Detect® spectrometer estimated the concentration of the Tetracosanoic Acid samples to be 0.853 ± 0.14 mg/mL (2.4%CV) and the CHAPS samples were estimated to be 1.8 ± 0.004% (2.3%CV).

Protein quantitation and lipid content analysis performed using the same measurement

IR spectral profiles of proteins and lipids are distinct, thereby enabling the simultaneous quantitation of proteins and analysis of lipids using the same measurement. Protein content can be quantified using one of the pre-loaded standard curves (all prepared in PBS pH 7.4)[2]. Lipid analysis can be performed, using the same spectra, in the "Relative Absorbance" mode. We analyzed phospholipids in a complex mixture (Figure 2). The protein in the sample was quantified at 2.8 mg/mL.

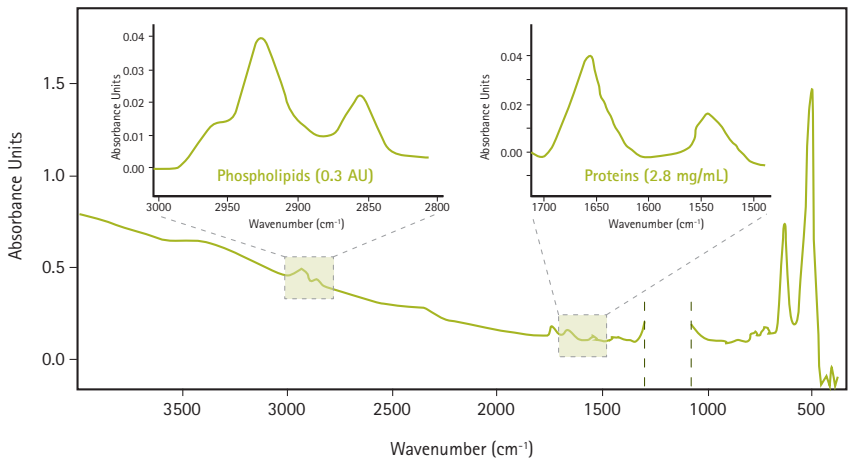


Figure 2. Analysis of protein and lipid content from a single sample measurement. Because the sample composition and ratio of individual components were unknown, the analysis of the phospholipids was performed using the "Relative Absorbance" mode.

Monitoring the lipid profile during the preparation of a breast cancer tissue lysate

Traditionally, quantitation of proteins and lipids in lysates has been tedious, requiring large sample volumes and specialized methodologies, and the results for either component obtained using classical methods may be obscured by cross-interference. The Direct Detect® spectrometer has enabled rapid analysis of total protein with simultaneous monitoring of lipid content, thereby simplifying and improving the analytical process[3]. Breast cancer tissue lysates were prepared using RIPA buffer and CytoBuster™ protein extraction reagent. Following tissue homogenization, the Direct Detect® spectrometer was used to monitor the efficiency of fat removal and total protein concentration during centrifugal extraction. Direct Detect® spectrometer data showed gradual removal of a fatty fraction from the samples (Figure 3). The same spectra were also used to determine the total protein recovery across the various fractions (Table 1). From this limited study, it is clear that the Direct Detect® system offers a means for in-line process optimization for maximal yield and/or purity.

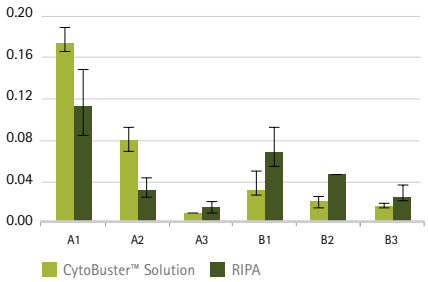


Figure 3. Lipid content as determined using the Direct Detect® spectrometer in the "Relative Absorbance" mode in 3 different fractions of breast cancer tissue lysates prepared with CytoBuster™ Protein Extraction Reagent or RIPA buffer.

Conclusions

The Direct Detect® spectrometer enables rapid analysis of lipids and detergents in addition to accurate and reproducible protein quantitation. The ability to simultaneously monitor protein concentration and fat removal during sample preparation provides a tool for assay optimization as well as greater confidence in final sample purity.

References
1. Pidgeon C., Apostol G. and Markovich R. Anal. Biochem. 1989, 181(1): 28-32.
2. Merck Millipore. Literature No. AB3355EN00, 2012.
3. Gutierrez, S. et al. Poster Presentation, Human Proteome Organization (HUPO) 11th Annual World Congress, Boston, MA, USA, September 2012.

Sample	Spin condition & fraction collected	Total protein content (mg/mL) CytoBuster™ Protein Extraction Reagent	RIPA
A1	spin @10,000 xg (top fatty fraction)	5.0	14.0
A2	spin @15,000 xg (top fatty fraction)	2.7	20.0
A3	spin @15,000 xg (bottom layer)	5.0	17.0
B1	spin @10,000 xg (top fatty fraction)	3.3	3.6
B2	spin @15,000 xg (top fatty fraction)	2.1	5.0
B3	spin @15,000 xg (bottom layer)	2.8	5.0

Table 1. Total protein recovery from breast cancer tissue lysed with RIPA buffer or CytoBuster™ protein extraction reagent measured using the Direct Detect® spectrometer.

For Direct Detect® spectrometer ordering information, see page 114.

Direct Detect® Spectrometer

IR-based protein quantitation

What if you never had to run another Bradford or BCA, ever? The Direct Detect® system provides more accurate results without the pitfalls of colorimetric assays. By measuring amide bonds in protein chains, the system accurately quantitates an intrinsic component of every protein without relying on amino acid composition, dye binding properties or reduction-oxidation (redox) potential. You can evaluate major components of complex mixtures separable from the Amide I & II region, like lipids and carbohydrates—making lysates and membrane preps easier than ever!

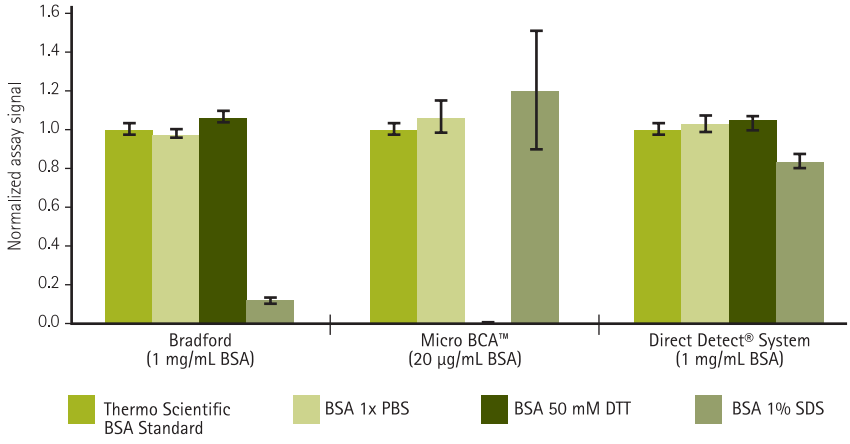
Features & Benefits

- IR spectrometry measures amide bond absorbance for more reproducible quantitation across all proteins and peptides
- Improves accuracy over traditional colorimetric assays
- More compatible with detergents, reducing agents and other buffer components that interfere with traditional protein quantitation assays, providing more information from your sample
- Preserves precious samples—requires only 2 µL per analysis
- New software module can now quantify long aliphatic chains in lipids and detergents

Applications

Protein Quantitation, Lipid Analysis, Peptide Quantitation

Product Performance



Direct Detect® spectrometer works where Bradford and BCA assays fail. The Direct Detect® spectrometer provides accurate and precise results, even in the presence of detergent (SDS) and reducing agent (DTT). Using the Direct Detect® system, calculated concentrations for all BSA samples matched the prediluted Thermo Scientific BSA Standard. In comparison, the Coomassie® Plus (Bradford) Assay results differed greatly in the presence of 1% SDS, and the Micro BCA™ assay could not provide data in the presence of 50 mM DTT.

Specifications

Quantitation Approach	Assay Range	Sample Volume (tube/plate)
Direct Detect® System	250-5,000 µg/mL	2 µL
Bradford Assay	20-2,000 µg/mL	50 µL/25 µL
Micro BCA™ Assay	2-4 µg/mL	500 µL/150 µL
Lowry Assay	10-1,500 µg/mL	200 µL/40 µL

Ordering Information

Description	Qty/Pk	Catalogue No.
Direct Detect® Spectrometer and Starter Kit	1	DDHW00010-WW
Includes:		
Direct Detect® Spectrometer	1	
Universal Power Adapter	1	
Dell Latitude® 2120 Netbook and Power Adapter	1	
Direct Detect® Software	1	
Netbook Stand	1	
Spotting Tray	1	
Ethernet Cable	1	
Direct Detect® Assay-free Cards (50/pk)	1	
Consumables		
Direct Detect® Assay-free Cards (50/pk)	1	DDAC00010-GR
Direct Detect® Assay-free Cards (50/pk)	4	DDAC00010-4P
Direct Detect® Assay-free Cards (50/pk)	8	DDAC00010-8P
Direct Detect® Desiccant Pack	5	DDSP00010-DE

For more information visit: www.merckmillipore.com/directdetect

Immobilon® Membranes, Sandwiches and Blotting Filter Paper

PVDF transfer membranes for Western blotting

Immobilon® PVDF membranes are offered in three types, each optimized for a different protein blotting application. Convenient blotting sandwiches feature pre-cut sheets of membrane and blotting filter paper. Immobilon® PVDF membranes have high protein adsorption, so you won't lose proteins during transfer or reprobing. The open pore structure makes it easy to access bound proteins and remove unbound probes. In addition, Immobilon® PVDF membranes optimized for fluorescent blots dramatically increase signal-to-noise ratios for high sensitivity in quantitative, multiplexing applications.

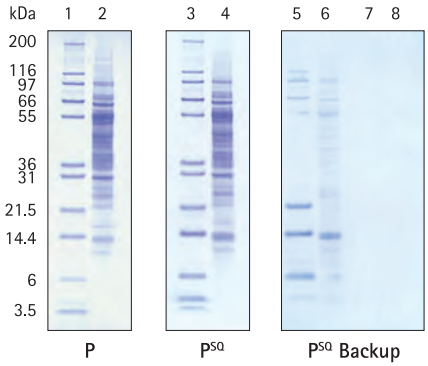
Features & Benefits

- Won't crack, curl or fracture when cut
- Low background
- Superior staining capabilities
- Can be reprobed multiple times

Applications

Western Blotting, Dot Blotting, Protein Sequencing; Compatible with Radioactive, Chromogenic, Chemiluminescent, Fluorescent, and Chemifluorescent Detection

Product Performance



Immobilon®-PSQ membrane prevents the proteins from blowing through the membrane, increasing protein signal. Molecular weight standards (lanes 1 and 3) and calf liver lysate (lanes 2 and 4) were transferred to Immobilon®-P or Immobilon®-PSQ membranes. A sheet of Immobilon®-PSQ membrane was placed behind the primary membranes to capture proteins that passed through (lanes 5 and 6 behind Immobilon®-P membrane; lanes 7 and 8 behind Immobilon®-PSQ membrane).

Specifications

	Immobilon®- P Membrane	Immobilon®-P^{SQ} Membrane	Immobilon®-FL Membrane
Filter Material	Hydrophobic PVDF	Hydrophobic PVDF	Hydrophobic PVDF
Filter Pore Size, µm	0.45	0.2	0.45
Protein Binding Capacity, µg/cm²			
Insulin	160	262	155
BSA	215	340	205
Goat IgG	294	448	300

Ordering Information

Description	Filter Dimensions (cm x cm)	Qty/Pk	Catalogue No.
Immobilon®-P PVDF Transfer Membrane, 0.45 µm			
Immobilon®-P Transfer Membrane	7 x 8.4	50	IPVH07850
	8 x 10	10	IPVH08100
	8.5 x 13.5	10	IPVH08130
	9 x 12	10	IPVH09120
	10 x 10	10	IPVH10100
	15 x 15	10	IPVH15150
	20 x 20	10	IPVH20200
	26 x 26	10	IPVH304F0
Immobilon®-P Transfer Membrane Roll	26.5 x 375	1	IPVH00010
Immobilon®-P^{SQ} PVDF Transfer Membrane, 0.2 µm			
Immobilon®-P^{SQ} Transfer Membrane	7 x 8.4	50	ISEQ07850
	8 x 10	10	ISEQ08100
	8.5 x 13.5	10	ISEQ08130
	9 x 12	10	ISEQ09120
	10 x 10	10	ISEQ10100
	15 x 15	10	ISEQ15150
	20 x 20	10	ISEQ20200
	26 x 26	10	ISEQ26260
Immobilon®-P^{SQ} Transfer Membrane Roll	26.5 x 375	1	ISEQ00010
Immobilon®-FL Membrane, 0.45 µm for Fluorescent Westerns			
Immobilon®-FL Transfer Membrane	10 x 10	10	IPFL10100
	20 x 20	10	IPFL20200
	26.5 x 375	1	IPFL00010
	7 X 8.4	10	IPFL07810
Immobilon® Blotting Sandwiches			
Immobilon®-P Blotting Sandwich	7 x 8.4	20	IPSN07852
	8.5 x 13.5	20	IPSN08132
Immobilon®-P Blotting Filter Paper	7 x 8.4	100	IBFP0785C
	8.5 x 13.5	100	IBFP0813C

For more information visit: www.merckmillipore.com/westernblotting

Detection: Fluorescent Westerns
Immobilon®-FL transfer membrane

Fluorescence-based detection of Western blots, while increasing in popularity due to multiplex detection capabilities, requires specialized tools to obtain optimal results. Merck Millipore's solutions for fluorescent Westerns, including Immobilon®-FL membrane, are designed to work together for fast, reproducible protein detection.

Publications citing Immobilon®-FL: ~9,000

How does Immobilon®-FL membrane work?

This 0.45 µm membrane is the first transfer membrane specifically optimized for fluorescence-based detection of Western blots. Its extremely low background autofluorescence improves sensitivity of all fluorescence detection protocols.

Key Benefits

- The only membrane that works at near-infrared wavelengths (700-800 nm)
- Strong signals due to higher protein adsorption & retention on the membrane
- Low background to detect even faint bands
- High tensile strength for multiple stripping and reprobing cycles

For more information, visit:
www.merckmillipore.com/flwestern

For product ordering information, see page 115.

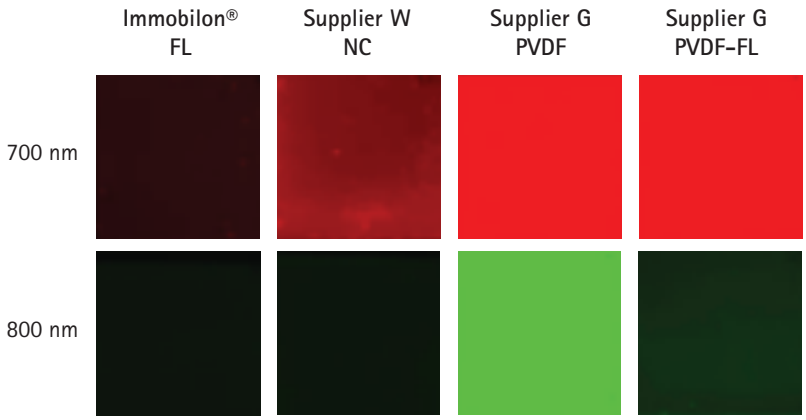


Figure 1. Lowest background fluorescence. Compared to nitrocellulose (NC) and PVDF membranes from other suppliers, Immobilon®-FL membrane exhibits the lowest background fluorescence at both wavelengths.

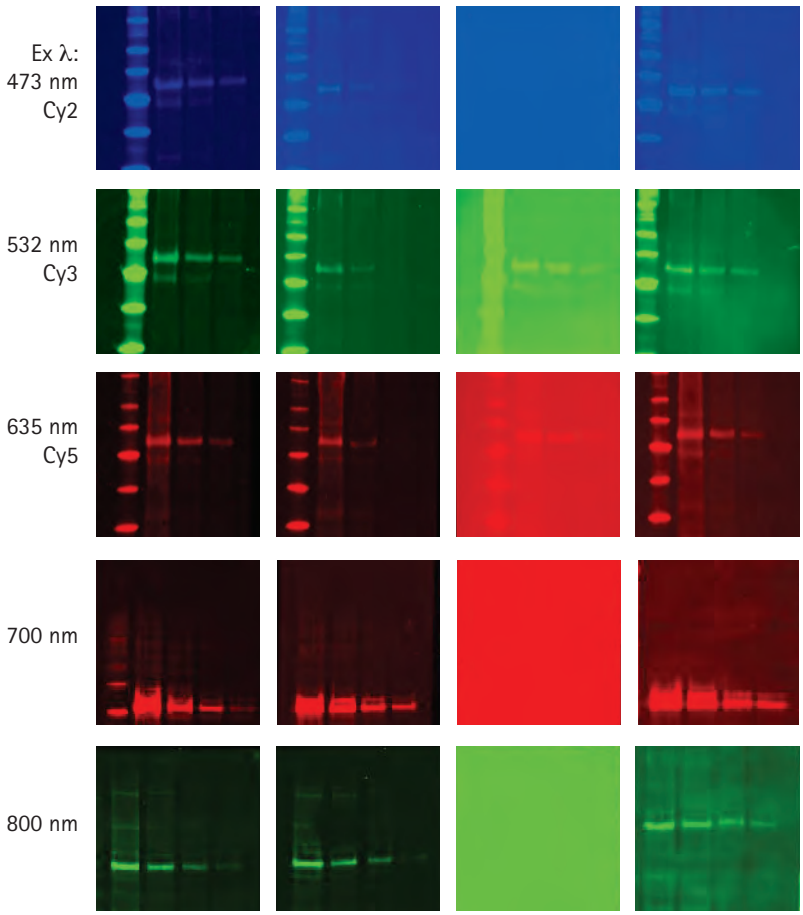


Figure 2. Highest sensitivity. Compared to other membranes, Immobilon®-FL membrane enables detection of smaller amounts of protein at all wavelengths, including near-infrared.

SNAP i.d.® 2.0 Protein Detection System

Rapid system takes protein detection to new dimensions

Unlike conventional Western blotting, where diffusion is the primary means of reagent transport, the SNAP i.d.® 2.0 system uses a vacuum to actively drive reagents through the membrane, enabling thorough washing and reducing the blocking, antibody incubation and wash steps to 30 minutes total. The SNAP i.d.® 2.0 system adds exciting new capabilities for Western blotting and requires no additional reagent consumption (e.g., antigen, antibody or detection reagents). The system's unique design enables the use of small volumes for antibody incubations with either polyvinylidene difluoride (PVDF) or nitrocellulose blotting membranes.

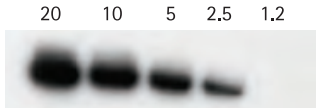
Features & Benefits

- Superior: Increased antibody-antigen binding, enhanced washes, and antibody recollection
- Flexible: Two gel sizes, mini (7.5 x 8.4 cm) and midi (8.7 x 13.5 cm)
- Fast: 30-minute immunodetection

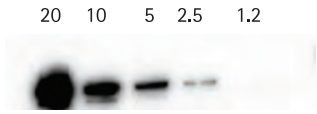
Applications

Western Blotting using Nitrocellulose or PVDF Membrane; Compatible with Radioactive, Chromogenic, Chemiluminescent, Fluorescent, and Chemifluorescent Detection

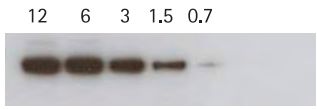
Product Performance



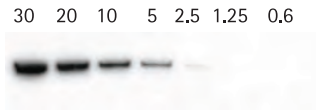
Anti-Huntingtin Protein (Catalogue No. MAB2166)
1:400 dilution of this antibody detected Huntingtin protein in rat brain lysate (20 - 1.2 µg).
Proteins were detected using Luminata™ Forte HRP detection reagent. Exposure of the blots to X-ray film time varies from 20 sec. to 30 min.



Anti-Metabotropic Glutamate Receptor 5 (Catalogue No. AB5675)
1:200 dilution of this antibody detected Metabotropic Glutamate Receptor 5 in rat brain lysate (20 - 1.2 µg).
Proteins were detected using Luminata™ Forte HRP detection reagent. Exposure of the blots to X-ray film time varies from 20 sec. to 30 min.



Anti-erbB2 (intracellular domain) (Catalogue No. 04-291)
1:200 dilution of this antibody detected erbB2 in A431 lysate (12 - 0.7 µg).
Proteins were detected using Luminata™ Forte HRP detection reagent and blots. Exposure of the blots to X-ray film varies from 20 sec. to 30 min.



Anti-Pyk2 (Catalogue No. 06-559)
1:200 dilution of this antibody detected Pyk2 protein in rat brain lysate (30 - 0.6 µg).
Proteins were detected using Luminata™ Forte HRP detection reagent and blots. Exposure of the blots to X-ray film varies from 20 sec. to 30 min.

The SNAP i.d.® 2.0 protein detection system enables sensitive, specific detection of diverse targets, using various antibodies.

Ordering Information

Description	Dimensions (cm)	Qty/Pk	Catalogue No.
SNAP i.d.® 2.0 Systems			
SNAP i.d.® 2.0 System-Mini	7.5 x 8.4	1	SNAP2MINI
SNAP i.d.® 2.0 System-Midi	8.5 x 13.5	1	SNAP2MIDI
SNAP i.d.® 2.0 System-Mini and Midi		1	SNAP2MM
SNAP i.d.® 2.0 Consumables			
SNAP i.d.® 2.0 Mini Blot Holders	7.5 x 8.4	100	SNAP2BHMN0100
SNAP i.d.® 2.0 Midi Blot Holders	8.5 x 13.5	100	SNAP2BHMD0100
SNAP i.d.® 2.0 Accessories			
SNAP i.d.® 2.0 Antibody Collection Tray		20	SNAPABTR
SNAP i.d.® 2.0 Blot Roller		1	SNAP2RL
SNAP i.d.® 2.0 Mini Blot Holding Frames		2	SNAP2FRMN02
SNAP i.d.® 2.0 Midi Blot Holding Frames (double pack)		2	SNAP2FRMD02
SNAP i.d.® 2.0 Mini Blot Holding Frame (single pack)		1	SNAP2FRMN01
SNAP i.d.® 2.0 Midi Blot Holding Frame (single pack)		1	SNAP2FRMD01
Accessories			
Chemical Duty Pump, 100 V/50-60 Hz		1	WP6110060
Chemical Duty Pump, 115 V/60 Hz		1	WP6111560
Chemical Duty Pump, 220 V/60 Hz		1	WP6122050
Vacuum Filtering Flask, 1 L		1	XX1004705
No. 8 Perforated Stopper, silicone		1	XX1004708
Filter Forceps, blunt end, stainless steel		3	XX6200006P

For more information visit: **www.merckmillipore.com/westernblotting**

Antibody recovery and reuse in the SNAP i.d.® 2.0 immunodetection system

Introduction

Antibody reuse for Western blotting is a common practice. While many antibodies lose potency with time or degrade even faster due to improper storage conditions, there is high potential value of recovering the primary antibody for possible reuse in some experiments.

Here, we compare antibody recovery and reuse in the standard immunodetection protocol with the antibody recovery and reuse in the SNAP i.d.® 2.0 system using the extended protocol and the original SNAP i.d.® protocol.

1. Assemble blot in blot holder/frame
2. Add blocking solution
3. Apply vacuum until completely dry
4. Turn vacuum off
5. Remove blot holder frame
6. Wipe any residual liquid at the bottom of the frame
7. **Place antibody collection tray**
8. Add primary antibody and incubate for 10 min or more
9. After incubation, turn vacuum on and wait for 1 min
10. Turn vacuum off and remove collection tray
11. Turn vacuum on for washing
12. Continue with the SNAP i.d.® protocol

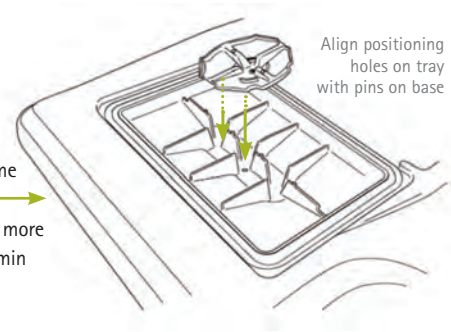


Figure 1. SNAP i.d.® 2.0 protocol, including antibody collection.

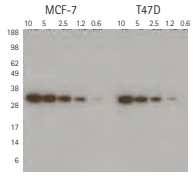
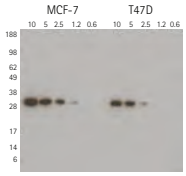
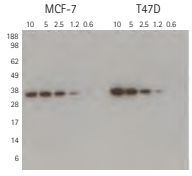
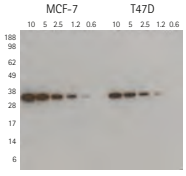
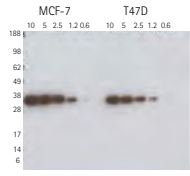
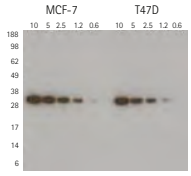
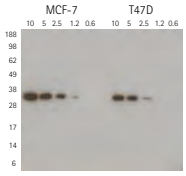
Method	Processing time	Blot processed with freshly diluted antibody	Blot processed with collected antibody	Processing time	Blot processed with antibody used for the third time
Standard/ Traditional Immunodetection Method	Blocking = 1 h Primary Ab =1 h Secondary Ab =1h 6 washes (5 min each) = ½ h **Total = 3.5 h**				
SNAP i.d.® Extended Protocol (Extended incubation of primary Ab only)	Blocking = 20 s Primary Ab = 1 h Secondary Ab =10 min 8 washes (20 s each) = 2 min 40 s **Total = 1 h 13 min**			Blocking = 20 s Primary Ab = Overnight Secondary Ab =10 min 8 washes (20 s each) = 2 min 40 s **Total = 14 hr 12 min**	
SNAP i.d.® Original Protocol	Blocking = 20 s Primary Ab = 10 min Secondary Ab =10 min 8 washes (20 s each) = 2 min 40 s **Total = 23 min**				

Figure 2. Two-fold dilution series of breast cancer cell lysates (MCF-7 and T47D, 10 to 0.6 µg total protein) were subjected to SDS-PAGE and transferred to blotting membranes in seven identical blots using three different immunodetection protocols as described above. The seven blots were probed with anti-PP2A, which was either freshly diluted, collected or recovered for a second time.

Conclusion

The SNAP i.d.® 2.0 Protein Detection System not only reduces Western blot processing time by as much as 80%, but it also conserves precious antibodies by providing a rapid and convenient method for the collection of primary antibodies for future reuse. Greater than 90% of the primary antibody volume can be recovered after the incubation step by following the recommended protocol. The antibodies collected can be used successfully in subsequent immunodetection with no reduction in blot quality, even in the extended protocol (1 h or overnight incubations).

For SNAP i.d.® 2.0 system ordering information, see page 118.

Western Blotting Reagents

Highest sensitivity with the least background

For simple, cost-effective protein detection, use our Western blotting reagents to achieve the highest sensitivity with the least background. Featured Western blotting tools include protein-free, room temperature-stable blocking reagents for chemiluminescent, fluorescent, and phosphoprotein detection and pre-mixed, room temperature-stable HRP substrates.

- Signal enhancing reagents amplify your signals so you can get your data more quickly and spend less time troubleshooting
- We also offer easy-to-use reagents for the removal of antibodies from Western blots that have been developed with chemiluminescent substrates

Features & Benefits

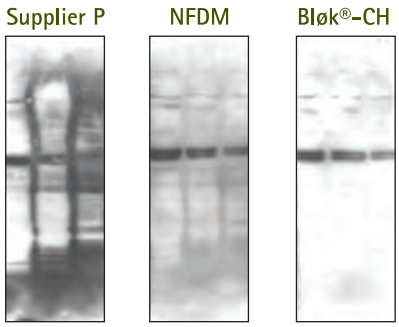
- Pre-optimized to work synergistically, providing strong, specific signals and low background

Applications

Western Blotting using Nitrocellulose or PVDF Membrane; Compatible with Radioactive, Chromogenic, Chemiluminescent, Fluorescent, and Chemifluorescent Detection

Product Performance

Bløk® reagents provide better signal-to-noise ratios compared to NFDM or blocking reagents from Supplier P. Chemiluminescence detection of p53 in EGF-stimulated A431 lysate (10-2.5 µg/lane). Blocking reagents used during the blocking and antibody incubation steps are indicated on top. NFDM = nonfat dry milk.



Ordering Information

Description	Reagents (Volumes)	Membrane Coverage (cm²)	Qty/Pk	Catalogue No.
Immobilon® Western Chemiluminescent HRP Substrate				
Immobilon® Western Chemiluminescent HRP Substrate	Luminol (25 mL); Peroxide solution (25 mL)	500	2 x 25 mL	WBKLS0050
	Luminol (50 mL); Peroxide solution (50 mL)	1000	2 x 50 mL	WBKLS0100
	Luminol (250 mL); Peroxide solution (250 mL)	5000	2 x 250 mL	WBKLS0500
Immobilon® Western AP Substrate				
Immobilon® Western AP Substrate, 25 mL		500	25 mL	WBKDS0025
Immobilon® Western AP Substrate, 100 mL		2000	100 mL	WBKDS0100
Bløk™ Noise Cancelling Reagents				
Bløk™- CH Noise Cancelling Reagents for Chemiluminescence Detection, 500 mL		1000	1	WBAVDCH01
Bløk™- FL Noise Cancelling Reagents for Fluorescent Detection, 500 mL		1000	1	WBAVDFL01
Bløk™- PO Noise Cancelling Reagents for Phosphoprotein Detection using Chemiluminescence or Fluorescence Techniques, 500 mL		1000	1	WBAVDP001

Ordering Information – Continued

Description	Membrane Coverage (cm^2)	Qty/Pk	Catalogue No.
Luminata™ Western HRP Substrates			
Luminata™ Classico Western HRP Substrate, 100 mL	1000	100 mL	WBLUC0100
Luminata™ Classico Western HRP Substrate, 500 mL	5000	500 mL	WBLUC0500
Luminata™ Crescendo Western HRP Substrate, 100 mL	1000	100 mL	WBLUR0100
Luminata™ Crescendo Western HRP Substrate, 500 mL	5000	500 mL	WBLUR0500
Luminata™ Forte Western HRP Substrate, 100 mL	1000	100 mL	WBLUF0100
Luminata™ Forte Western HRP Substrate, 500 mL	5000	500 mL	WBLUF0500
ReBlot™ Plus Reagents			
ReBlot™ Western Blot Recycling Kit		1 kit	2060
ReBlot™ Plus Kit		1 kit	2500
ReBlot™ Plus Mild Antibody Stripping Solution, 10x		50 mL	2502
ReBlot™ Plus Strong Antibody Stripping Solution, 10x		50 mL	2504

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- Availability of both human and mouse angiogenesis/growth factor panels

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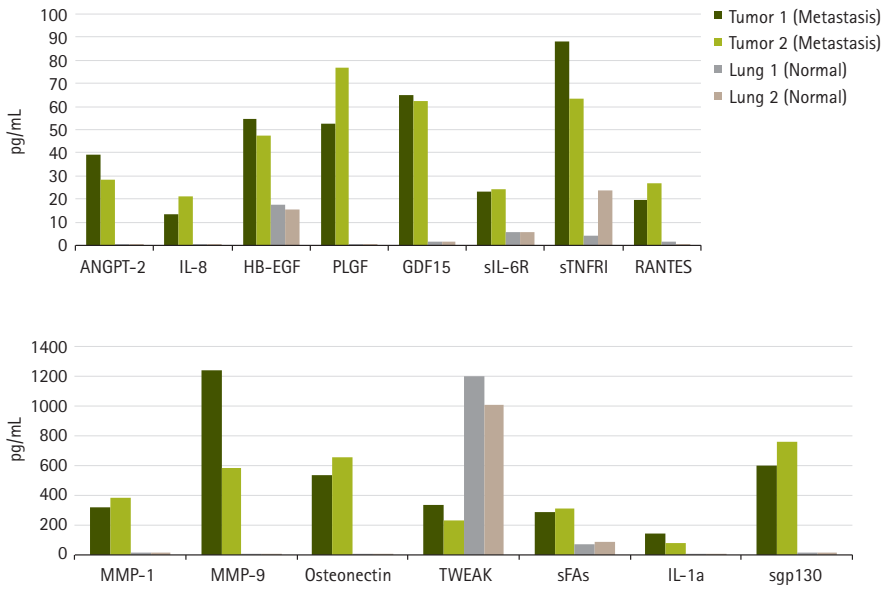

Identification of tumor-associated biomarkers (cell migration, tumor invasion, angiogenesis and metastasis) in lung tissue lysates derived from tumor-bearing and control (normal) mice. Lung tissue samples harvested 38 days after tumor inoculation and normal lung was harvested from age-matched controls. MILLIPLEX® MAP Human Cancer, Angiogenesis, Metastasis, Cytokine, Bone, MMP and TIMPs Panels were used to identify biomarker candidates.

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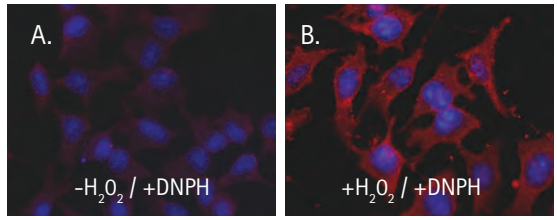

Oxidative stress detection with OxyBlot™, OxyELISA™, OxyICC™ (shown), IH, and flow cytometry assays. Under physiological and pathophysiological oxidative stress, oxygen free radicals and other reactive species result in carbonyl groups being introduced into protein side chains. Merck Millipore's oxidative stress detection kits enable simple and sensitive immunodetection of these carbonyl groups, following specific modification with dinitrophenylhydrazine (DNPH). DNPH-modified carbonyls (red) are more abundant in peroxide-treated cells (B), compared to untreated cells (A).

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