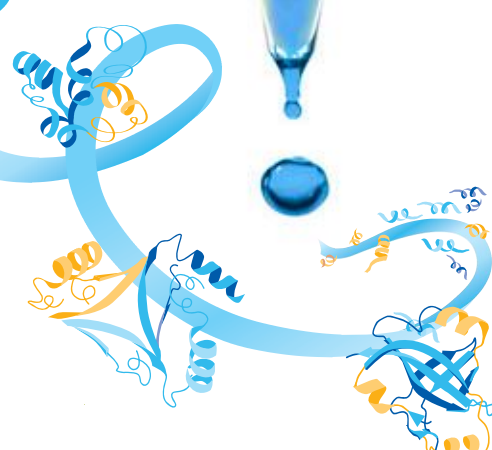


Protein purification
and preparation.
High purity and
recovery for
better discovery.



Introduction

Today, researchers are challenged to create high quality protein samples for successful proteome analysis, often using cumbersome traditional sample preparation methods. With over 50 years of experience in developing protein sample preparation technologies, Merck Millipore is constantly innovating new tools to offer you rapid and efficient solutions that can be smoothly integrated into your workflow.

Why spend your time on arduous sample preparation protocols when you can focus your efforts on exciting experiments? With the right pure protein, in the buffer you need, at the concentration you want, your next discovery is only a step away. From protein isolation to purification, you can count on Merck Millipore to support your research. To learn more, please visit: www.merckmillipore.com/psp

Key Features

Unmatched Flexibility

Isolate proteins from a diverse range of sample types with our flexible, broad range of kits.

Multiple downstream applications

Our kits enable you to produce samples that can be used directly in applications such as activity assays, protein microarrays, SDS-PAGE, immunoblotting, ELISA, two-dimensional gel electrophoresis (2DGE), mass spectrometry (MS; including MS/MS, LC-MS, MALDI-MS, SELDI-MS, and ESI-MS), and others.

Scale-up compatibility

It's easy to scale up to high-throughput recombinant protein purification and solubility screening using our sample preparation reagents.

Protein Extraction 4



Protein Extraction with Cell Lysis Reagents ("Busters") 6

Fast, simple, gentle protein extraction from *E. coli*, yeast, insect and mammalian cells

Cell Lysis and Nucleic Acid Removal Enhancers 8

Increase your protein extraction yield and purity with Benzonase® and rLysozyme™ reagents

Protein Extraction with ProteoExtract® Kits 10

Extract proteins from different fractions of the cell, including membrane, nucleus, cytosol and cytoskeleton (mammalian cells only)

Protein Extraction with Inhibitors 12

Protease and phosphatase inhibitor cocktails to prevent proteolysis and dephosphorylation of your proteins

Protein Purification 14



Affinity Purification with PureProteome™ Magnetic Beads 15

Ideal for small volume applications such as immunoprecipitation, depletion, recombinant protein screening, etc.

Agarose-Based Affinity Purification 18

Protein A, Protein G, His•Tag®, GST•Tag™, S•Tag™ and other fusion tags

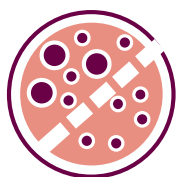
Amicon® Pro Purification System 22

Purify, buffer exchange and/or concentrate in one device

Protease Cleavage Enzymes 25

Recombinant enterokinase, factor Xa, HRV 3C protease, thrombin and other enzymes for cleaving fusion proteins

Protein Buffer Optimization and Sample Concentration 27



Dialysis and Buffer Exchange Devices 27

Amicon® Pro Purification System, D-Tube™ Dialyzers and Amicon® Ultra Diafiltration for protein sample desalting and buffer exchange

Centrifugal Concentration Devices 31

Amicon® Ultra filters for fast and effective protein concentration

Specialized Concentration Devices 35

Microcon®, Ultrafree® and Centriprep® filters for efficient purification, concentration, and desalting of biological samples

Clinical Filtration Devices 38

Centrifree® and Minicon® concentrators for concentration or partition of body fluids or other biological specimens

Large Volume Concentration Devices 40

Centricon® Plus-70 for rapid processing of aqueous biological solutions in larger volumes; stirred cells and cut discs for concentrating volumes up to 400 mL



Protein Extraction

When purifying proteins for functional or structural studies, the first step is to disrupt the cells or tissue sample and extract the relevant protein fraction. This step is critical because processing methods that require harsh mechanical, chemical, or enzymatic treatments can affect the target protein's integrity and

activity, or otherwise expose it to degradative conditions. Merck Millipore's complete range of reagents and enzymes for cell lysis and protein extraction provide you with an array of options so that you can put together the perfect extraction protocol for your particular cells and protein.

Protein Extraction Reagents Application Guide

| Products by Cell Type | Starting Material | | Applications | | | Comments |
|--|-------------------|-------------|--------------|---------------|----------------|--|
| | Total Culture | Cell Pellet | 1D PAGE | 2D PAGE / IEF | Activity Assay | |
| E. COLI | | | | | | |
| 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® Protein Extraction Reagent | | • | • | • | • | Efficient protein extraction from <i>E. coli</i> under non-denaturing conditions. |
| 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. |
| PopCulture® Reagent | • | | • | | • | Protein extraction from cells directly in the culture medium; no centrifugation required. |
| 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. |
| Insect PopCulture® Reagent | • | | • | | • | Lysis of insect cells directly in serum-free medium. Ideal for expression screening of many small samples. |
| 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. |
| ProteoExtract® Kits | | • | • | •* | • | Extract protein fractions from different parts of the cell. A range of kits offering maximum flexibility. |
| LYSIS AND EXTRACTION ENHANCEMENT | | | | | | |
| Gram-negative bacteria (<i>E. coli</i>) | | | | | | |
| rLysozyme™ Solution | • | • | • | | • | Cleaves bond in peptidoglycan layer of <i>E. coli</i> cell wall. |
| Lysonase™ Bioprocessing Reagent | • | • | • | | • | Convenient mixture of rlysozyme™ 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 chromatography, and reduced interference in assays. |

1D PAGE – One-dimensional Polyacrylamide Gel Electrophoresis

2D PAGE – Two-dimensional Polyacrylamide Gel Electrophoresis

IEF – Isoelectric Focusing

* – Salt must be removed before IEF

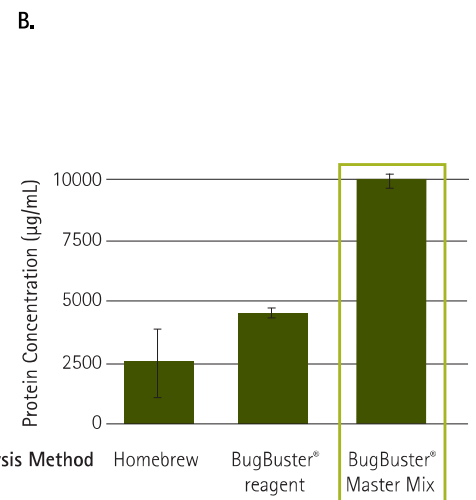
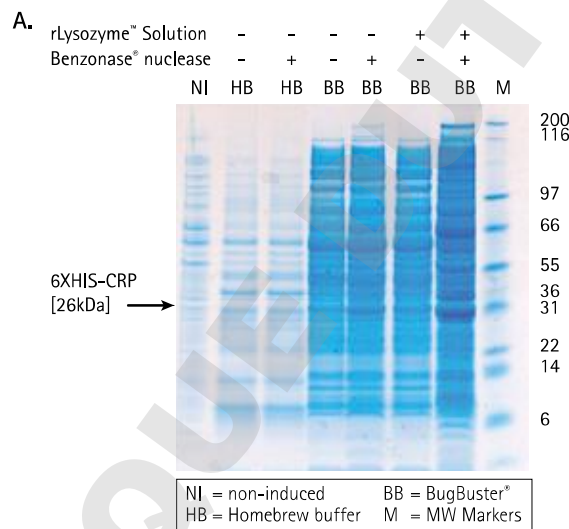
Protein Extraction with Cell Lysis Reagents ("Busters")

Featured Products

BugBuster® Protein Extraction Kits and Reagents

Simple extraction of soluble protein from *E. coli* without sonication

Gently disrupt the cell wall of *E. coli* and liberate soluble proteins with BugBuster® Kits and Reagents. BugBuster® reagent provides a simple, rapid, low-cost alternative to mechanical methods such as French press or sonication for releasing expressed target proteins in preparation for purification or other applications. The proprietary formulation uses a detergent mix to perforate cell walls without denaturing soluble protein. Simply harvest cells by centrifugation and suspend in BugBuster® reagent. Following a brief incubation, remove insoluble cell debris by centrifugation. The clarified extract is ready to be purified.



BugBuster® reagent is superior to "Homebrew" lysis buffer and BugBuster® reagent with both Benzonase® nuclease and rLysozyme™ solution yielded lysates with the most 6XHIS-CRP.

(A) *E. coli* lysates (5 µL of 1 mL total lysate) from various lysis protocols were fractionated and analyzed by SDS-PAGE. A band corresponding to 6XHIS-CRP is prominently visualized in the BB +/+ lane. (B) Cleared cell lysates (2 µL of 1 mL total) were spotted on assay cards and quantified using the Direct Detect® spectrometer. In each case, bars represent the average of 3 independent samples.

*The Direct Detect® system is an infrared-based quantitation platform from Merck Millipore (Catalog No. DDHW00010-WW). Visit www.merckmillipore.com/directdetect for details.

How do I choose between BugBuster® Products?

Components of Bacterial Lysis Reagents

| | BugBuster® Reagent | Buffer | Benzonase® Nuclease | rLysozyme™ Solution | Notes |
|-------------------------------------|-----------------------|--------|------------------------|------------------------|--|
| BugBuster® Reagent | X | X | | | |
| BugBuster® 10X | X | | | | Flexibility to customize dilution and buffer composition |
| BugBuster® Plus Benzonase® Nuclease | X | X | X | | 2 separate vials for greater flexibility |
| BugBuster® Plus Lysonase™ Kit | X | X | X | X | 2 separate vials for greater flexibility |
| BugBuster® Master Mix | X | X | X | X | 1 convenient reagent |
| PopCulture® Reagent | X | X | | | Buffer protects protein from the pH extremes produced in high density culture media, enabling extraction directly in medium. |

We offer a family of protein extraction reagents for gentle, efficient, non-mechanical extraction of soluble proteins from bacteria, yeast, plant, mammalian, and insect cells.

CytoBuster™ reagent – Obtain protein extracts from mammalian and insect cells in their native state in 5 minutes.

NucBuster™ reagent – Extract nuclear proteins in less than 30 minutes with a simple 2 step protocol.

PhosphoSafe™ Extraction reagent – The PhosphoSafe™ Extraction Buffer is a detergent and Phosphatase inhibitor mixture optimized for fast, efficient extraction of soluble proteins from mammalian and insect cell preserving the phosphorylation state of your sample.

YeastBuster™ reagent – Extract proteins from yeast and plants without mechanical disruption and enzymatic lysis.

The reagent has been tested with *Saccharomyces cerevisiae*, *Pichia pastoris*, *P. stipidis*, and *Schizosaccharomyces pombe* strains and with plant cells.

Insect PopCulture® reagent – Insect PopCulture® Reagent is a detergent-based lysis reagent specifically formulated for total insect cell culture (in suspension or adherent) extraction without the need for centrifugation.

Ordering Information

Available from www.merckmillipore.com

| Application | Description | Catalog No. |
|-------------|---|-------------|
| Bacteria | BugBuster® Protein Extraction Reagent | 70584 |
| | BugBuster® Master Mix | 71456 |
| | BugBuster® Plus Benzonase® Nuclease | 70750 |
| | BugBuster® Plus Lysonase™ Kit | 71370 |
| | BugBuster® 10X Protein Extraction Reagent | 70921 |
| | PopCulture® Reagent | 71092 |
| Mammalian | CytoBuster™ Protein Extraction Reagent | 71009 |
| | NucBuster™ Protein Extraction Reagent | 71183 |
| | PhosphoSafe™ Extraction Reagent | 71296 |
| Yeast | YeastBuster™ Protein Extraction Reagent | 71186 |
| Insect | Insect PopCulture® Reagent | 71187 |

Cell Lysis and Nucleic Acid Removal Enhancers

Featured Products

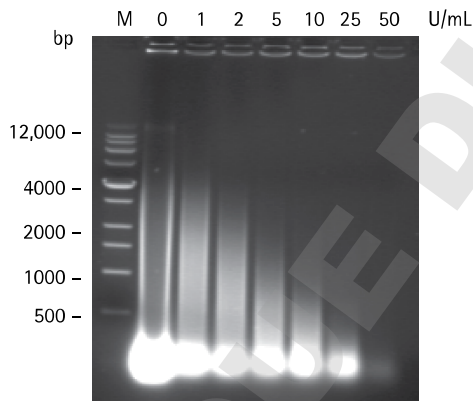
Benzonase® Nuclease

Effectively reduce viscosity and remove nucleic acids from protein solutions

Benzonase® Nuclease is a genetically engineered endonuclease from *Serratia marcescens*. It degrades all forms of DNA and RNA (single stranded, double stranded, linear and circular) while having no proteolytic activity. It is effective over a wide range of conditions and has an exceptionally high specific activity. Benzonase® nuclease is an excellent choice for viscosity reduction to shorten processing time and increase yields of protein.

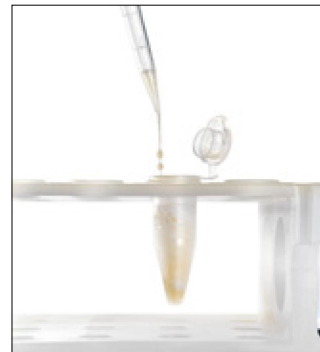
Benzonase® Advantages

- Compliant with FDA guidelines for nucleic acid contamination
- Functional between pH 6 and 10, from 0 °C to 42 °C for maximum versatility
- Active in the presence of ionic and non-ionic detergents, reducing agents, PMSF (1 mM), EDTA (1 mM) and urea.
- Available in ultrapure (> 99% by SDS-PAGE) and pure (> 90%) grades
- Available in standard concentration (25 U/μL) and high concentration (HC, 250 U/μL).



Nucleic acid digestion by Benzonase® Nuclease.

E. coli BL21(DE3) cells containing a pET construct were suspended in BugBuster® Reagent (5 mL/g wet weight). Identical volumes of the suspension were treated with the indicated amounts of Benzonase® Nuclease for 30 min at room temperature. Samples were clarified by centrifugation and analyzed by agarose gel electrophoresis and ethidium bromide staining.



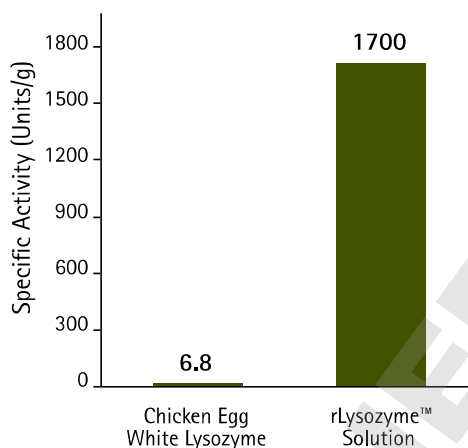
E. coli lysate without Benzonase® Nuclease.

Goey, viscous, difficult to handle.

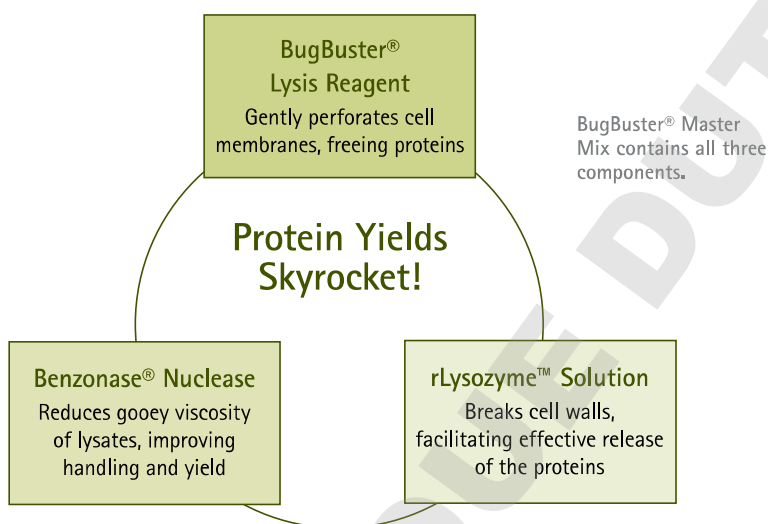
rLysozyme™ Solution

Degrade bacterial cell walls with stabilized recombinant lysozyme

rLysozyme™ Solution contains a highly purified and stabilized recombinant lysozyme that can be used for lysis of *E. coli*. The enzyme catalyzes the hydrolysis of N acetylmuramide linkages in bacterial cell walls. The specific activity of rLysozyme™ (1700 KU/mg) for *E. coli* lysis is 250 times greater than that of traditional chicken egg white lysozyme. rLysozyme™ is optimally active at physiological pH. Very small amounts of rLysozyme™ enhance the efficiency of protein extraction with BugBuster® and PopCulture® Reagents. The product is supplied as a ready-to-use solution and is stable at -20 °C.



rLysozyme™ exhibits 250 times higher specific activity than chicken egg white activity when measured using a standard activity assay.



Ordering Information

Available from www.merckmillipore.com

| Description | Catalog No. |
|-------------------------------------|-------------|
| Benzonase® Nuclease, Purity >90% | 70746 |
| Benzonase® Nuclease HC, Purity >90% | 71205 |
| Benzonase® Nuclease, Purity >99% | 70664 |
| Benzonase® Nuclease HC, Purity >99% | 71206 |
| rLysozyme™ Solution | 71110 |
| Chicken Egg White Lysozyme Solution | 71412 |
| Lysonase™ Bioprocessing Reagent | 71230 |



Protein Extraction with ProteoExtract® Kits

Featured Products

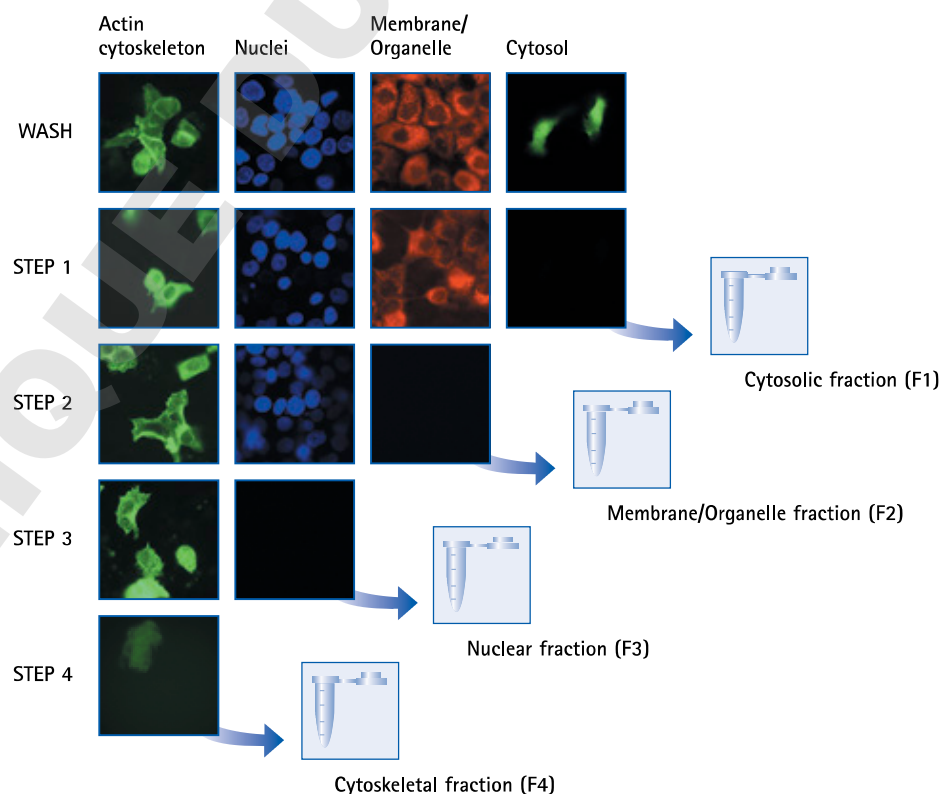
ProteoExtract® Subcellular Proteome Extraction Kit (S-PEK)

Reproducible extraction of subcellular proteomes from mammalian cells.

Based on different solubilities of certain subcellular compartments, the S-PEK uses proprietary chemistries to yield four subproteome fractions which are enriched in cytosolic, membrane/organelle, nuclear, and cytoskeletal proteins. In the case of adherent cells, the procedure is performed directly in the tissue culture dish without the need for cell removal. For suspension-grown cells, extraction starts with gentle sedimentation and washing of cells. Extraction from tissues requires isolation of viable cells before proceeding with the extraction protocol.

Applications of S-PEK:

- Subcellular redistribution assays to monitor protein translocation
- Enzyme activity assays including reporter gene assays and kinase assays
- SELDI (surface-enhanced laser desorption/ionization)-profiling
- Non-denaturing gel electrophoresis
- Assaying protein expression levels using fluorescent-labeled subcellular extracts in microarrays



Four distinct protein fractions separated using S-PEK. A431 cells were incubated with DAPI (nuclei), phalloidin (to stain actin) and MitoTracker® probes, 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.

ProteoExtract® Native Membrane Protein Extraction Kit (M-PEK)

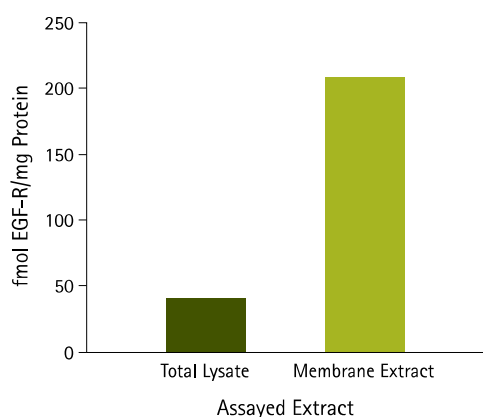
Isolation of native membrane proteins from mammalian cells and tissue.

Extract proteins associated with cellular membranes with M-PEK. The extremely mild extraction conditions yield a 3-5 fold enrichment of integral membrane and membrane-associated proteins. The simple, two-step procedure enables processing of multiple samples in parallel. Extraction from tissues requires isolation of viable cells before proceeding with the extraction protocol.

Applications for Extracted Membrane Proteins:

- Enzyme activity assays, including reporter gene assays and kinase assays
- Non-denaturing and denaturing gel electrophoresis, immunoblots and immunoassays
- Assaying post-translational modifications, such as phosphorylation
- SELDI-profiling of integral and membrane-associated proteins
- NHS ester labeling of membrane proteins

EGF-Receptor Enrichment



Greatly increased enrichment of EGF receptor using M-PEK compared to total cell lysate. HEK293 cells were extracted with buffered 1% Triton® X-100 surfactant to generate a total lysate or extracted with M-PEK to yield a membrane fraction. Equal volumes of these fractions were utilized to quantitate the concentration of EGF receptor in the samples using a EGF-R ELISA Kit. Protein concentrations were used to calculate the amount of EGF-R per mg protein in the total lysate and the membrane fraction. The measurements demonstrate a 4.5 fold enrichment of the EGF receptor in the M-PEK-extracted membrane fraction.

Ordering Information

Available from www.merckmillipore.com

| Application | Description | Catalog No. |
|------------------------------|---|-------------|
| Organelle Fractionation | ProteoExtract® Subcellular Protein Extraction Kit | 539790 |
| | ProteoExtract® Complete Mammalian Protein Extraction Kit | 539779 |
| | ProteoExtract® Cytosol/Mitochondria Fractionation Kit | QIA88 |
| | ProteoExtract® Native Cytoskeleton Enrichment Kit | 17-10210 |
| | ProteoExtract® Cytoskeleton Enrichment and Isolation Kit | 17-10195 |
| Membrane Proteins | ProteoExtract® Native Membrane Protein Extraction Kit | 444810 |
| | ProteoExtract® Transmembrane Protein Extraction Kit | 71772 |
| Mass Spec Peptide Enrichment | ProteoExtract® All-in-One Trypsin Digestion Kit | 650212 |
| | ProteoExtract® Glycopeptide Enrichment Kit | 72103 |
| | ProteoExtract® Phosphopeptide Enrichment TiO ₂ Kit | 539722 |
| Albumin and IgG Depletion | ProteoExtract® Albumin Removal Kit | 122640 |
| | ProteoExtract® Albumin/IgG Removal Kit | 122642 |

Protein Extraction with Inhibitors

Featured Products

Protease Inhibitor Cocktails

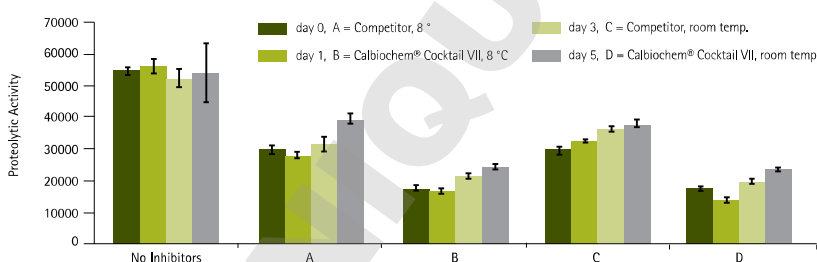
Prevent protein degradation by proteases during extraction and purification

Ensure the integrity of purified proteins by using protease inhibitor cocktails and highly specific protease inhibitors. During protein expression and isolation, endogenous proteases rapidly begin to degrade protein samples, reducing the quality and quantity of protein samples required for characterization and analysis. By using the right combination of protease inhibitors, you can protect your purified protein preparations from common proteases including serine proteases, metalloproteases, cysteine proteases, aminopeptidases, and aspartic proteases.

Protease Inhibitor Advantages:

- Convenient—Flexible protocol and ready-to-use formulations
- Consistent—High quality ensures reproducibility and excellent inhibition over a wide range of protease classes
- Flexible—Comprehensive selection of specific cocktail formulations designed to inhibit proteolytic activity from most tissue or cell type extracts, including mammalian, bacterial, yeast, fungal, and plant cells
- Application-Specific—Available without EDTA for purification schemes involving metal ion chelation
- Chromatography or analysis using 2D gel electrophoresis. New protease inhibitor cocktail formulations include recombinant aprotinin for applications that require the use of animal-free reagents

Calbiochem® Protease Inhibitors offer greater efficiency and stability



Stability of Protease Inhibitor Dilutions in BugBuster® Lysis Reagent. Protease inhibitors were diluted to the prescribed working concentration (Competitor or Calbiochem® Cocktail VII, Cat. No. 539138). The ability of the inhibitors to inhibit the proteolytic activity of PRONASE® reagent (Cat. No. 537088) was measured by using the Universal HT Protease Assay on days zero, one (24 h post dilution), three (72 h) and five (120 h). The Universal HT Protease Assay quantifies protease activity using a fluorescein thiocarbonyl-casein derivative (FTCcasein). Proteolytic activity liberates FTC-labeled peptides, which results in enhanced fluorescence (Ex,max: 495 nm; Em,max: 525 nm). Addition of the protease inhibitor cocktails inhibits the proteolytic activity of the PRONASE® reagent (Cat. No. 537088), resulting in reduced fluorescence. On day 1, for samples incubated at 8 °C, the competitor tablet inhibited the proteolytic activity by 50% and the Calbiochem® Cocktail VII inhibited the proteolytic activity 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 in proteolytic activity. The data show that the efficiency of the cocktail remained higher than the competitor's tablet in this study.

Featured Protease Inhibitor Cocktails

Protease Inhibitor Cocktail Set III, EDTA-Free (Cat. No. 539134)

This popular cocktail is widely cited in publications, and has been used in multiple applications, such as Western blot, immunoprecipitation, kinase assay and ubiquitination assay. This cocktail is recommended for use with mammalian cell and tissue extracts and is also suitable for bacterial cell extracts for metal chelation chromatography. It contains six protease inhibitors (in 1 mL DMSO) with broad specificity for the inhibition of aspartic, cysteine, and serine proteases as well as aminopeptidases. Each vial contains the concentrations of inhibitors shown in the table below. One mL is sufficient for about 20 g tissue.

Visit www.merckmillipore.com/inhibitors for a complete listing of our inhibitor cocktails.

Phosphatase Inhibitor Cocktails

Prevent protein dephosphorylation for cell signaling studies

It is critical to preserve the phosphorylation state of proteins of interest during their extraction from cell and tissue lysates. To effect cell signaling, target proteins are phosphorylated by protein kinases that transfer a phosphate group to specific sites, typically at serine, threonine, or tyrosine residues. These phosphate groups can be removed by protein phosphatases, restoring the protein to its original dephosphorylated state. Using phosphatase inhibitors help reveal the signaling status inside a cell at a specified timepoint. Merck Millipore offers four different Phosphatase Inhibitor cocktails and a PhosphoSafe™ Extraction Reagent that help protect phosphoproteins from different families of phosphatases.

Featured Phosphatase Inhibitor Cocktail

Phosphatase Inhibitor Cocktail Set II (Cat. No. 524625)

This cocktail of five phosphatase inhibitors for the inhibition of acid and alkaline phosphatases as well as protein tyrosine phosphatases (PTPs) is widely cited and has been used, for example, in studies of EGFR signaling, apoptosis pathways and inflammation.

Suitable for use with tissue and cell extracts, including extracts containing detergents. Each vial contains 1 mL aqueous solution of the phosphatase inhibitor cocktail.

The concentrations of the individual inhibitors are shown in the table below. Note: 1 set = 5 x 1 mL.

Ordering Information

Available from www.merckmillipore.com

| Description | Recommended Application | Catalog 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 2-D 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 2-D 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 | 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 Protein Tyrosine Phosphatases (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 Protein Tyrosine Phosphatases (PTPs) | 71296 |

Visit www.merckmillipore.com/inhibitors for a complete listing of our inhibitor cocktails.



Protein Purification

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. Merck Millipore offers a wide range of tools for protein purification, including affinity magnetic beads, affinity agarose resins, Amicon® Pro purification system and protease cleavage enzymes.

- PureProteome™ magnetic beads are ideal for small volume affinity purification assays, such as immunoprecipitation and serum depletion or enrichment.
- Affinity agarose portfolio for larger volume applications, such as antibody purification and recombinant protein purification.
- Amicon® Pro purification system is ideal for small volume affinity purification assays followed by buffer exchange and/or concentration.
- Protease cleavage enzymes available in restriction grade or in kits for cleaving fusion proteins.



Agarose Portfolio

| Application | Magnetic | Agarose | Amicon® Pro System |
|------------------------------------|--|--|--------------------|
| IP and Antibody Purification | Protein A Protein G Kappa Ig Binder Lambda Ig Binder | Protein A Protein G Protein G/Protein A | ✓ |
| Recombinant Tag Purification | His•Tag® purification | His•Tag® purification GST•Tag™ purification S•Tag™ purification Strep •Tag® II purification T7•Tag™ purification | ✓ |
| Protease Cleavage | | Thrombin Factor Xa Enterokinase HRV 3C Protease | |
| Biotinylated Molecule Purification | Streptavidin | Streptavidin | ✓ |
| Depletion/Enrichment | Albumin Albumin/IgG Depletion Kit Human Albumin/Ig Depletion Kit | ProteoExtract® Albumin Kit ProteoExtract® Albumin/IgG Kit | ✓ |
| Custom Labeled | NHS FlexiBind Carboxy FlexiBind | | ✓ |

Affinity Purification with PureProteome™ Magnetic Beads

PureProteome™ Protein A and G Beads Fast and easy immunoprecipitation

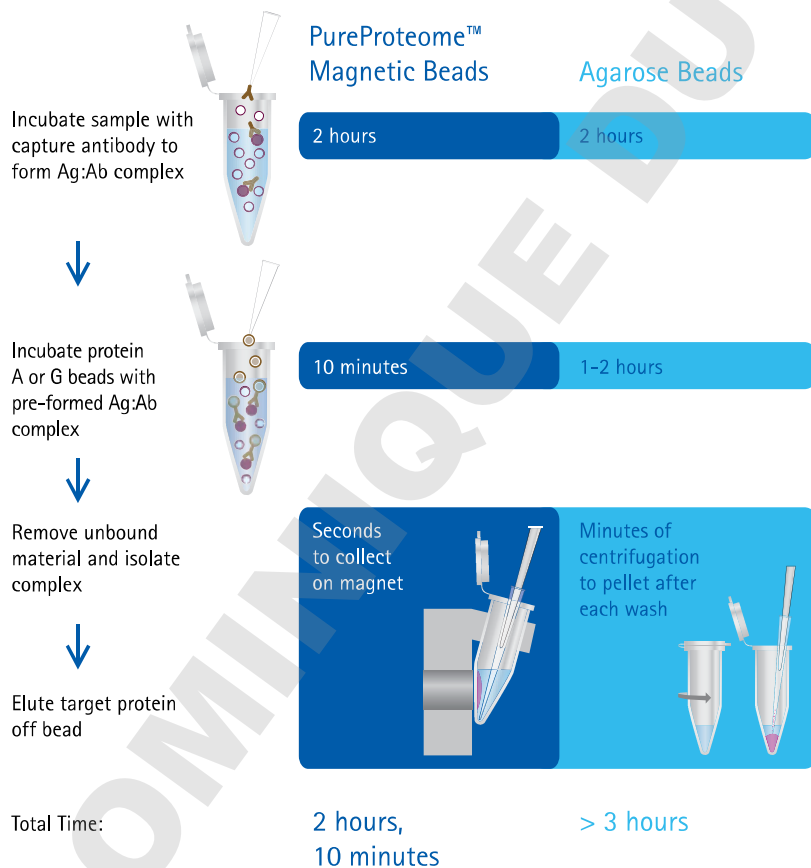
Traditional methods require hours of incubation time and minutes of harsh centrifugation to isolate sample. In contrast, PureProteome™ magnetic beads enhance binding equilibrium, enabling faster, gentler processing. The beads are easily resuspended for fast mixing and efficient interaction between the beads and protein.

PureProteome™ Protein A/G Mix Beads

Bind all mammalian immunoglobulin G (IgGs) efficiently using PureProteome™ Protein A/G mix magnetic beads, which provide a 50:50 blend of Protein A and Protein G.

Advantages of PureProteome™ Immunoprecipitation:

- Be efficient with high capacity beads: increased surface area allows for significantly greater binding capacity than non-Merck Millipore beads
- Achieve high purity: low non-specific binding of other proteins
- Save time with fast sample processing: enhanced binding equilibrium decreases incubation times by > 50%

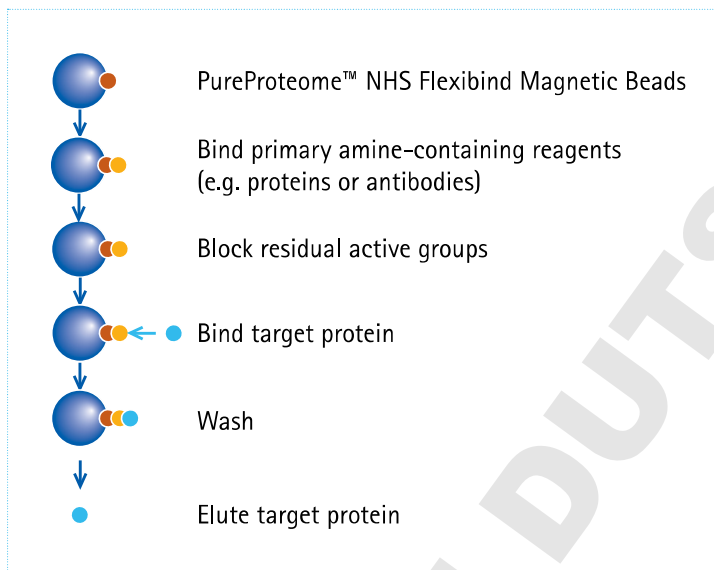


High speed immunoprecipitation with magnetic beads compared to agarose. In parallel indirect immunoprecipitations, PureProteome™ magnetic beads offered a 50% reduction in incubation time while yielding results equivalent to agarose beads.

PureProteome™ NHS and Carboxy FlexiBind beads

Customize your beads quickly and easily

Tailor your beads to match your application. Studying protein-protein interactions? Immobilizing enzymes, nucleic acids or small molecules? PureProteome™ NHS and Carboxy FlexiBind magnetic beads offer you flexibility in binding your target ligand. To customize your bead, the only requirement is that your target ligand has a free amine group.



- **Flexibility:** Choose from a range of sizes and chemistries to fit your application
- **Speed:** Get results faster
- **Cost Savings:** Less sample and reagent waste

PureProteome™ NHS FlexiBind Magnetic Beads (perfect for the first time user)

- **Fast:** Customize your own bead in <60 min
- **Easy to Use:** Kit contains everything you need: beads, all buffers and Amicon® Ultra centrifugal filters for eliminating unreacted species
- **Robust:** Little experience or optimization required

PureProteome™ Carboxy FlexiBind Magnetic Beads (for the experienced user)

- **Flexible:** Choice of 0.3 µm, 1 µm or 2.5 µm COOH magnetic beads
- **Automation-Compatible:** Smaller beads have higher buoyancy properties while retaining strong magnetic capability
- **Economical:** Aggressive pricing

PureProteome™ Kappa and Lambda Ig Binder beads

Immunoprecipitate all Human Antibodies (including IgA, IgD, IgE and IgM)

PureProteome™ Kappa Magnetic Beads bind to the kappa light chain constant region on human immunoglobulins with high specificity, and the Lambda Magnetic Beads bind to the lambda light chain constant region on human immunoglobulins with high specificity. These novel magnetic beads are capable of capturing all immunoglobulin subtypes (IgG, IgA, IgD, IgE, and IgM) and provide a rapid, scalable, and reproducible means to capture human antibody or antibody fragments containing kappa or lambda light chains – including Fab and F(ab')₂.

Depletion of all human immunoglobulins can be performed by mixing PureProteome™ Kappa and Lambda Magnetic Beads.

Relative Affinity

| Antibodies | Protein A/G Mix | Protein A | Protein G | Kappa Ig Binder | Lambda Ig Binder | Kappa/Lambda mix* |
|-------------------------|-----------------|-----------|-----------|-----------------|------------------|-------------------|
| Rabbit IgG | ● | ● | ● | | | |
| Mouse IgM | ● | ● | | | | |
| Mouse IgG ₃ | ● | ● | ● | | | |
| Mouse IgG _{2b} | ● | ● | ● | | | |
| Mouse IgG _{2a} | ● | ● | ● | | | |
| Mouse IgG ₁ | ● | ● | ● | | | |
| Human IgM | ● | ● | | ● | ● | ● |
| Human IgE | ● | ● | | ● | ● | ● |
| Human IgD | ● | ● | | ● | ● | ● |
| Human IgA | ● | ● | | ● | ● | ● |
| Human IgG ₄ | ● | ● | ● | ● | ● | ● |
| Human IgG ₃ | ● | ● | ● | ● | ● | ● |
| Human IgG ₂ | ● | ● | ● | ● | ● | ● |
| Human IgG ₁ | ● | ● | ● | ● | ● | ● |
| Rat IgM | ● | ● | | | | |
| Rat IgG _{2c} | ● | ● | ● | | | |
| Rat IgG _{2b} | ● | ● | ● | | | |
| Rat IgG _{2a} | ● | ● | ● | | | |
| Rat IgG ₁ | ● | ● | ● | | | |
| Rat IgG | ● | ● | ● | | | |

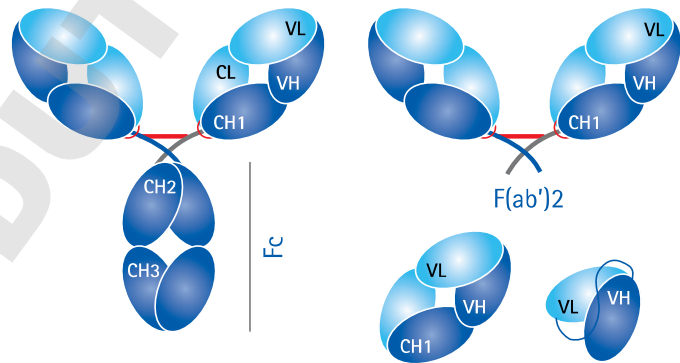
* PureProteome™ Kappa/Lambda mix is not a catalog item. Simply procure the Kappa and Lambda beads individually and mix at a 1:1 ratio.

| Fragments | A/G Mix | Protein A | Protein G | Kappa Ig Binder | Lambda Ig Binder | Kappa/Lambda mix* |
|---------------------------|---------|-----------|-----------|-----------------|------------------|-------------------|
| Human λ | | | | | ● | ● |
| Human κ | | | | ● | | ● |
| Human Fc | ● | ● | ● | | | |
| Human scFv | ● | ● | | ● | ● | ● |
| Human F(ab') ₂ | ● | ● | ● | ● | ● | ● |
| Human Fab | ● | ● | ● | ● | ● | ● |

Key code for relative affinity of protein A and G; PureProteome™ Kappa and Lambda magnetic beads for respective antibodies:

- Strong affinity
- Moderate/slight affinity
- Requires evaluation

PureProteome™ Kappa or Lambda light chain ligands bind to the constant region of the antibody light chain, so PureProteome™ Kappa or Lambda ligands will not bind scFv.



Ordering Information

Available from www.merckmillipore.com/psp

| Application | Description | Catalog No. |
|--|---|--------------|
| IP, antibody purification, Fab purification | PureProteome™ Protein A Magnetic Beads | LSKMAGA10 |
| | PureProteome™ Protein G Magnetic Beads | LSKMAGG10 |
| | PureProteome™ Protein A/G Mix Magnetic Beads | LSKMAGAG10 |
| | PureProteome™ Kappa Ig-Binder Magnetic Beads* | LSKMAGKP02 |
| | PureProteome™ Lambda Ig-Binder Magnetic Beads* | LSKMAGLM02 |
| Biotinylated molecule purification | PureProteome™ Streptavidin Magnetic Beads | LSKMAGT10 |
| His•Tag® tagged protein purification | PureProteome™ Nickel Magnetic Beads | LSKMAGH10 |
| Custom labelled (flexibility to bind ligand of choice) | PureProteome™ NHS FlexiBind Magnetic Beads | LSKMAGN04 |
| | PureProteome™ Carboxy FlexiBind Magnetic Beads** | LSKMAG1CBX10 |
| Depletion/Enrichment | PureProteome™ Albumin Magnetic Beads | LSKMAGL10 |
| | PureProteome™ Albumin/IgG Depletion Kit | LSKMAGD12 |
| | PureProteome™ Human Albumin/Immunoglobulin Depletion Kit* | LSKMAGHDKIT |
| Magnetic Stands | PureProteome™ Magnetic Stand, 8-well | LSKMAGS08 |
| | PureProteome™ Magnetic Stand, 15 mL | LSKMAGS15 |

* Human only.

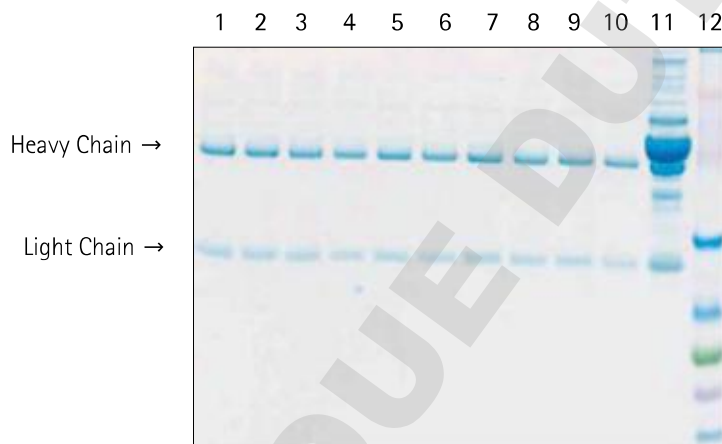
** Available in 0.3, 1.0 and 2.5 µM.

Agarose Based Affinity Purification

Agarose resins are the preferred approach for large purifications and a convenient option when scaling up will be needed. We offer a complete portfolio of agarose resins and kits for antibody purification, immunoprecipitation, and purification of tagged proteins.

Antibody Purification and Immunoprecipitation

Protein A and Protein G are proteins of microbial origin that bind specifically to mammalian immunoglobulins. When coupled to agarose, they provide an efficient tool for purification and immunoprecipitation of antibodies. Immunoglobulins of various species interact differently with the two proteins. A combination of Protein A and Protein G agarose is a good choice to have the characteristics of each in one reagent.



Montage® Antibody Purification Kits

From the initial clarification stage to the final antibody concentration step. High capacity pre-packed spin columns: no tedious chromatographic steps, no expensive hardware. Purify 10–20 mg in less than 60 minutes.

Human IgG Purifications/10X reuse with Human Serum. Human IgG was purified 10 consecutive times from normal serum using the regenerated Montage® spin column with PROSEP®-G media. An average of 12.96 mg of Human IgG was purified over 10 cycles with a CV of 7.3%.

Ordering Information

| Description | Size | Catalog No. |
|-------------------------------|--------|-------------|
| Protein A Agarose | 1.5 mL | IP02-1.5ML |
| | 10 mL | 16-125 |
| Protein A Agarose Fast Flow | 10 mL | 16-156 |
| Protein G Agarose | 1.5 mL | IP04-1.5ML |
| | 10 mL | 16-266 |
| Protein A + Protein G Agarose | 1.5 mL | IP05-1.5ML |
| | 10 mL | IP10-10ML |

| Description | Size | Catalog No. |
|---|------------------|-------------|
| Montage® Antibody Purification Kit with PROSEP®-A media | 20 purifications | LSK2ABA20 |
| Montage® Antibody Purification Kit with PROSEP®-G media | 20 purifications | LSK2ABG20 |

His•Tag® Purification

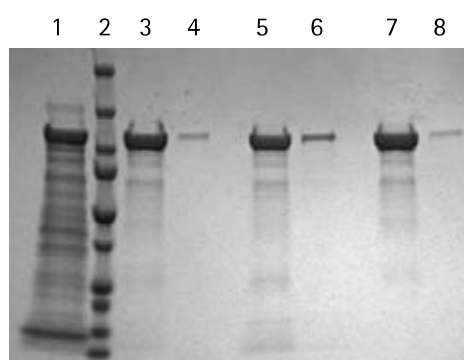
Purification is based on the affinity between the neighboring histidines of the His•Tag® sequence and an immobilized metal ion (usually Ni²⁺ or Co²⁺). The metal is held by chelation with reactive groups covalently attached to a solid support. The most commonly used chelators include nitriloacetic acid (NTA) and iminodiacetic acid (IDA).

NTA has an additional chelation site that minimizes leaching of the metal during the purification and has a broad chemical compatibility including reducing agents like 2ME.

Ni-NTA His•Bind® Resin is always an optimal choice and has a binding capacity over 10 mg of His-Tagged fusion protein per mL resin.

The agarose matrix on the Ni-NTA His•Bind® Superflow™ Resin has a higher level of crosslinking for higher bead rigidity making it compatible with FPLC.

Our IDA His•Bind® resins are offered uncharged to allow flexibility of choice in the metal ion (Nickel, Cobalt, Zinc, Iron, Copper, etc.). IDA supports can be recycled many times with no loss in performance.



← Target protein

Ni-NTA His•Bind® performance vs. equivalent competitor resins Vector pET-28b (+) was used to express a His-Tag fusion protein of 119KDa in *E. coli* BL21 (DE3) cells, induced culture was processed with BugBuster® Master Mix, and protein extract was divided evenly to proceed to the His-Tag purification using Ni-NTA His•Bind®, Ni-NTA Competitor Q, and Ni-NTA Competitor G resins. Ni-NTA His•Bind® resins show higher binding capacity and a better purification.

| Lane | Sample |
|------|-----------------------------|
| 1 | Crude Extract |
| 2 | Markers |
| 3 | Ni-NTA Competitor Q Elution |
| 4 | Ni-NTA Competitor Q Strip |
| 5 | Ni-NTA Competitor G Elution |
| 6 | Ni-NTA Competitor G Strip |
| 7 | Ni-NTA His•Bind® Elution |
| 8 | Ni-NTA His•Bind® Strip |

Ordering Information

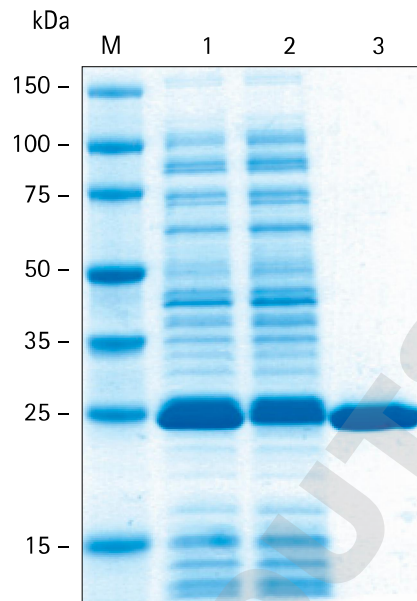
Available from www.merckmillipore.com/psp

| Application | Description | Catalog No. |
|--|--|-------------|
| Ni-NTA His•Bind® Resin | | |
| Small to medium scale | Ni-NTA His•Bind® Resin | 70666 |
| Gravity flow column | BugBuster® Ni-NTA His•Bind® Purification Kit | 70751 |
| Recommended for eukaryotic extracts | Ni-NTA Buffer Kit | 70899 |
| Ni-NTA His•Bind® Superflow™ Resin | | |
| Small to production scale | Ni-NTA His•Bind® Superflow™ Resin | 70691 |
| FPLC or gravity flow column | Ni-NTA Buffer Kit | 70899 |
| Uncharged IDA His•Bind® Resin | | |
| Uncharged (metal flexibility) | IDA His•Bind® Resin | 69670 |
| Reusability | His•Bind® Buffer Kit | 69755 |
| Small to medium scale | His•Bind® Purification Kit | 70239 |
| Gravity flow column or batch mode | BugBuster® His•Bind® Purification Kit | 70793 |

Affinity Purification with Recombinant Fusion Tags

GST•Tag™ Purification

The GST fusion system is based on the widely recognized affinity of glutathione-S-transferase (GST) fusion proteins for immobilized glutathione. Our GST Resin utilizes an 11-atom spacer arm to covalently attach reduced glutathione to the solid support via a sulfide linkage. The resin can be reused several times without loss of capacity and the high degree of substitution of glutathione ensures a high binding capacity.



| Lane | Sample |
|------|------------------------------------|
| M | PerfectProtein™ markers 15-150 kDa |
| 1 | BugBuster® extract |
| 2 | Flow-through |
| 3 | Eluate |

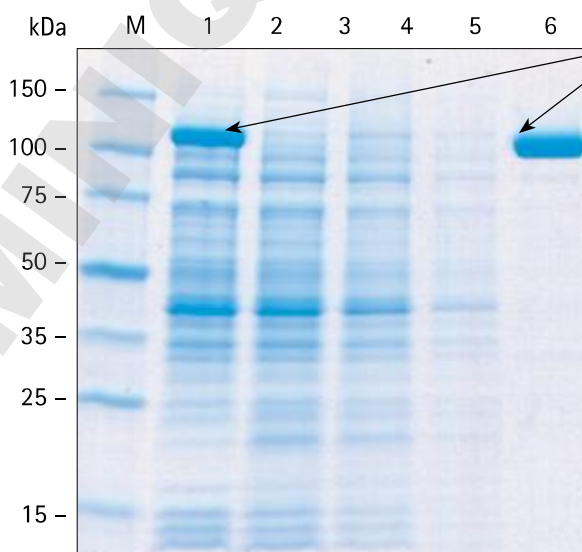
← Target protein

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.

S•Tag™ Purification

Featured Product

The S•Tag™ fusion protein is a short 15-aa sequence that specifically binds with high affinity the 104-aa S-Protein ($K_d=10^{-9}$ M, 1000 times stronger than the interaction between Nickel and His•Tag® fusion protein). Fusion proteins can be easily purified by cleavage with site specific proteases or in acidic buffers.



Target protein

| Lane | Sample |
|------|--|
| M | PerfectProtein™ markers 15-150 kDa |
| 1 | Crude extract |
| 2 | Flow-through |
| 3 | Wash 1 |
| 4 | Wash 2 |
| 5 | Eluate + Biotinylated Thrombin |
| 6 | Eluate after Biotinylated Thrombin removal |

S•Tag™ affinity purification

S•Tag™ β-gal expressed from a pET construct was purified from a crude soluble fraction using S-protein Agarose under native conditions. Elution of the target protein from the agarose was performed by digestion with Biotinylated Thrombin, which was subsequently removed with Streptavidin Agarose. The fractions are indicated.

Strep•Tag® II Purification

The Strep•Tag® fusion protein II is an 8 amino acid sequence that binds to the biotin pocket of Streptavidin with 100 times higher binding capacity.

T7•Tag® Purification

Purification is antibody-based. Covalently coupled to agarose beads, the T7•Tag® monoclonal antibody captures the T7•Tag® – a sequence of 11 amino acids.

Streptavidin Agarose

Cross-linked agarose is covalently coupled with pure streptavidin under controlled conditions. The stable linkage to the resin minimizes leaching of the streptavidin while maintaining full binding activity. The matrix is suitable for use in column and batch formats for any application that requires high biotin binding capacity and low non-specific binding and is ideal for affinity purification of biotinylated proteins or pull down experiments of biotinylated DNA/RNA probes. The resin has no detectable protease, DNase, or RNase.

Ordering Information

Available from www.merckmillipore.com/psp

| Description | Catalog No. |
|---------------------------------------|-------------|
| GST•Tag™ Purification | |
| GST•Bind™ Resin | 70541 |
| GST•Bind™ Buffer Kit | 70534 |
| BugBuster® GST•Bind™ Purification Kit | 70794 |
| S-Tag Purification | |
| S-protein Agarose | 69704 |
| S•Tag™ Thrombin Purification Kit | 69232 |
| S•Tag™ rEK Purification Kit | 69065 |
| Strep•Tag® II Purification | |
| Strep-Tactin® Superflow Agarose | 71592 |
| Strep-Tactin® Buffer Kit | 71613 |
| Strep-Tactin® SpinPrep Kit | 71608 |
| D-Desthiobiotin | 71610 |
| T7•Tag® Purification | |
| T7•Tag® Affinity Purification Kit | 69025 |
| T7•Tag® Antibody Agarose | 69026 |

| Description | Size | Catalog No. |
|----------------------|-------|-------------|
| Streptavidin Agarose | 5 mL | 69023-3 |
| | 10 mL | 16-126 |

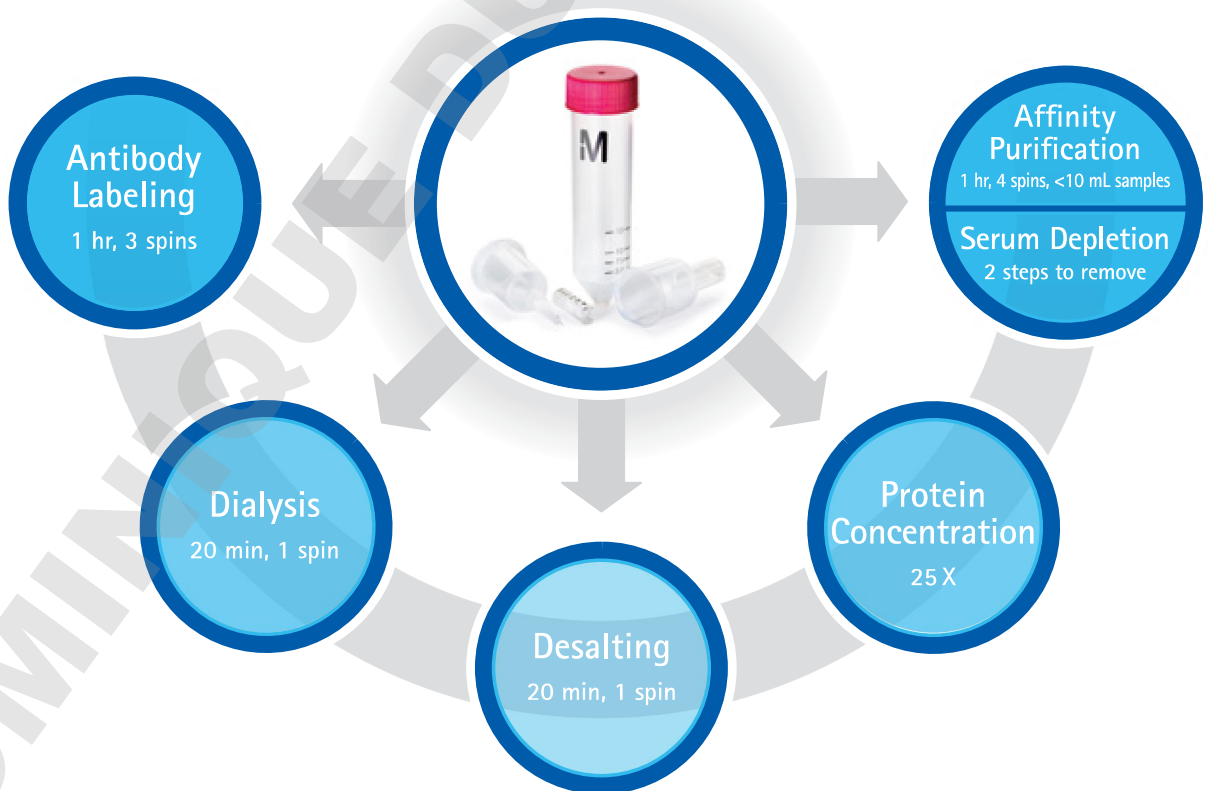
Amicon® Pro Purification System

Purify biologically active proteins with gentle, all-in-one recovery.

Biologically active proteins yield meaningful data. When you start with consistent yields of active, native-folded protein, you're giving your experiment the best chance to succeed. If your current protein preps involve juggling columns, dialyzers and multiple transfer steps, you could be introducing variability into your data. For your next protein preparation, choose the simple, gentle method that tackles even the most labile and poorly expressed proteins—the Amicon® Pro purification system. When your proteins behave, your research will flourish.



WORKING WITH PROTEINS?



A simple, flexible tool for the basic researcher.

Whether you're performing affinity purification from a precious sample, labeling antibodies, depleting abundant proteins from serum samples or removing salts from a chromatography sample, the Amicon® Pro system is your sample preparation partner. The modular design not only allows flexibility in application, but also offers unprecedented simplicity in protein sample preparation.

Examples:

- Turn your crude lysate into a purified, concentrated protein ready for your downstream assay in as few as four spins.
- Perform a 99% buffer exchange in a patent-pending, continuous, gentle process in one spin

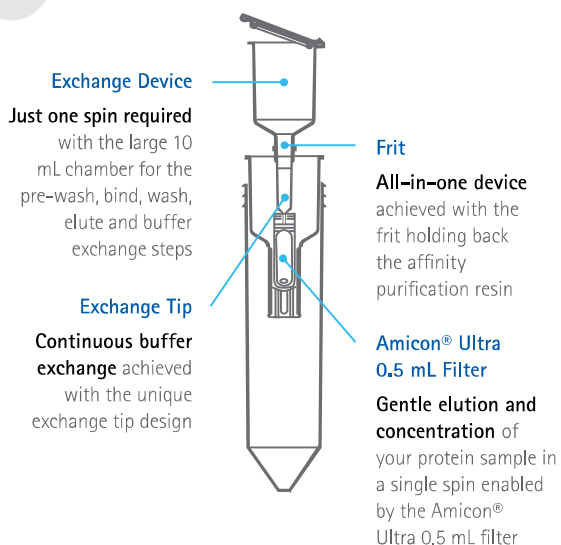
Don't lose protein in multiple devices.

Maximize your protein recovery with the Amicon® Pro System.

Traditional protein purification can be a long process with multiple steps and devices, which can often result in protein degradation and loss along the way. By using the Amicon® Pro Purification System, you can avoid the risks involved with sample transfer and you can reduce hands-on time.

Whether you need to affinity purify, concentrate, dialyze or any combination of the three, the Amicon® Pro Purification System will save you time and improve your protein recovery. It can help you perform multiple protein preparations in parallel, improving prep-to-prep reproducibility and enabling head-to-head comparison of expression constructs.

Amicon® Pro system unique design features and workflow benefits



"If I was doing things the old way, I would be six months—if not a year—behind where I am right now with my project."

—Jason Lehmann, Amicon® Pro user,
University of California in San Diego

Ordering Information

To choose the appropriate Amicon® Pro device, determine the molecular weight cut-off (MWCO) of your protein of interest and your desired affinity purification scheme. For convenience and ease of use, the Amicon® Pro purification kits contain devices, reagents and buffers optimized for twelve reactions. These kits are ideal for affinity purification of tagged recombinant proteins, antibody purification and depletion.

| Amicon® Pro Purification Kits 12/pk Includes reagent kit (resin and buffers) | Reagent Kit Only | MWCO | | | | |
|---|---------------------|-----------|-----------|-----------|-----------|-----------|
| | | 3,000 | 10,000 | 30,000 | 50,000 | 100,000 |
| Amicon® Pro Affinity Concentration Kit Ni-NTA | ACR5000NT | ACK5003NT | ACK5010NT | ACK5030NT | ACK5050NT | ACK5100NT |
| Amicon® Pro Affinity Concentration Kit Protein A | ACR5000PA | ACK5003PA | ACK5010PA | ACK5030PA | ACK5050PA | ACK5100PA |
| Amicon® Pro Affinity Concentration Kit Protein G | ACR5000PG | ACK5003PG | ACK5010PG | ACK5030PG | ACK5050PG | ACK5100PG |
| Amicon® Pro Affinity Concentration Kit GST | ACR5000GS | ACK5003GS | ACK5010GS | ACK5030GS | ACK5050GS | ACK5100GS |

| Amicon® Pro purification system – No Reagents Included | MWCO | | | | |
|---|-----------|-----------|-----------|-----------|-----------|
| | 3,000 | 10,000 | 30,000 | 50,000 | 100,000 |
| Amicon® Pro Purification System 12/pk | ACS500312 | ACS501012 | ACS503012 | ACS505012 | ACS510012 |
| Amicon® Pro Purification System 24/pk | ACS500324 | ACS501024 | ACS503024 | ACS505024 | ACS510024 |

What more will you discover using the Amicon® Pro system?

Find out at: www.merckmillipore.com/amiconpro

Protein Purification with Protease Cleavage Enzymes

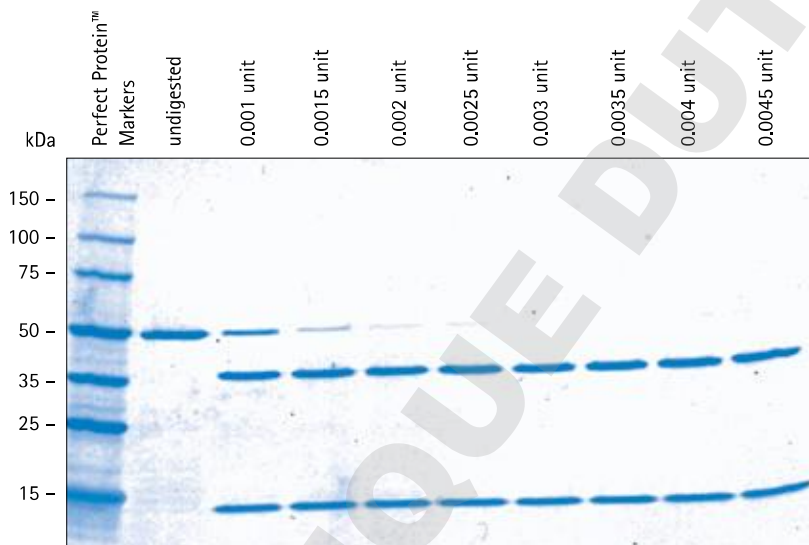
Featured Products

Restriction and Biotinylated Grade Thrombin

Highly efficient, specific cleavage of fusion proteins

Restriction Grade Thrombin is qualified to specifically cleave target proteins containing the recognition sequence LeuValProArg ↓ GlySer. The preparation is functionally tested for activity with fusion proteins and is free of detectable contaminating proteases. Thrombin is supplied with 10X Thrombin Cleavage Buffer and a Cleavage Control Protein.

Biotinylated Thrombin is identical in activity to Restriction Grade Thrombin, but has covalently attached biotin for easy removal of the enzyme from cleavage reactions using immobilized streptavidin. Our Thrombin Cleavage Capture Kit not only includes biotinylated thrombin and immobilized streptavidin but also all required buffers and filters for complete, convenient recovery of cleaved protein.



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.

HRV 3C Protease

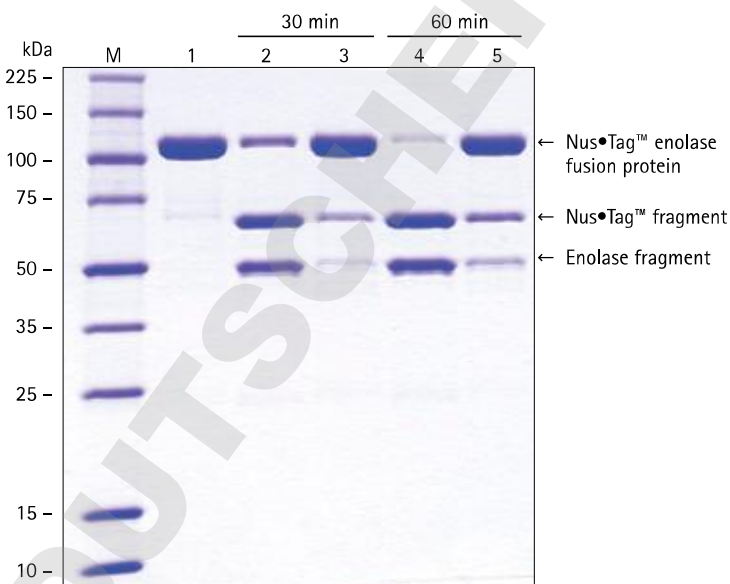
Highly efficient, specific cleavage of fusion proteins

Recombinant type 14 3C protease from human rhinovirus (HRV 3C) is a highly purified, recombinant 6XHis-tagged enzyme, which recognizes the cleavage site LeuGluValLeuPheGln ↓ GlyPro.

The small, 22-kDa size of the protease, with optimal activity at 4 °C, high specificity, and His-tag fusion make HRV 3C protease an ideal choice for rapid removal of fusion tags.

| Lane | Sample |
|------|---|
| M | PerfectProtein Markers, 10–225 kDa |
| 1 | 3 µg purified Nus•Tag™ enolase fusion protein |
| 2 | 3 µg Nus•Tag™ enolase fusion protein with 30-min HRV3C protease reaction |
| 3 | 3 µg Nus•Tag™ enolase fusion protein with 30-min competitor's protease reaction |
| 4 | 3 µg Nus•Tag™ enolase fusion protein with 60-min HRV3C protease reaction |
| 5 | 3 µg Nus•Tag™ enolase fusion protein with 60-min competitor's protease reaction |

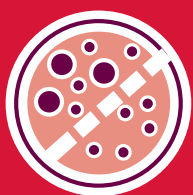
HRV 3C Protease cleaves fusion proteins more efficiently compared to cleavage with a competitor's protease. Using a 1:100 (w/w) ratio of protease:target protein, 500 µg of purified Nus•Tag™ enolase fusion protein was incubated in parallel 500 µL reactions at 4°C. The reactions was quenched by adding equal volume 4X SDS Sample Buffer and then immediately placing the samples into a water bath at 75 °C for 5 min.



Ordering Information

Available from www.merckmillipore.com

| Description | Catalog No. |
|-----------------------------------|-------------|
| Restriction-Grade Thrombin | 69671 |
| Biotinylated Thrombin | 69672 |
| Thrombin Cleavage Capture Kit | 69022 |
| Restriction Grade Factor Xa | 69036 |
| Factor Xa Cleavage Capture Kit | 69037 |
| Recominant Enterokinase | 69066 |
| Enterokinase Cleavage Capture Kit | 69067 |
| HRV 3C Protease | 71493 |
| Tag-off™ High Activity rEK | 71537 |
| Tag-off™ rEK Cleavage Capture Kit | 71540 |



Protein Buffer Optimization and Sample Concentration

When downstream quality matters, make sure your upstream tools are the best. The last steps of preparing a protein sample for downstream analyses, such as activity assays or structural studies, involve ensuring that the protein is in its native, soluble form, dissolved in the buffer of choice, and at an appropriate concentration. With Merck Millipore's tools for protein buffer optimization and sample concentration, obtain publication-quality data from every last microgram of protein.

Protein Buffer Exchange, Sample Desalting, and Dialysis

Each protein preparation is unique. Give it the special treatment it deserves with a perfectly designed device for dialyzing and buffer exchange. Select between fast and gentle diafiltration using the Amicon® Pro System or dialysis using D-Tube™ Dialyzers.

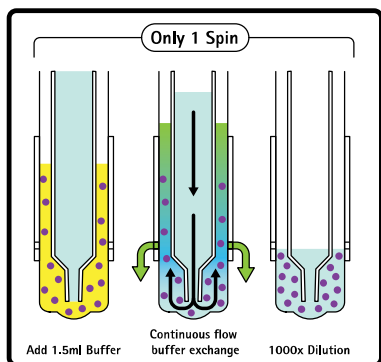


| Sample Needs | Amicon® Pro System | Amicon® Ultra Filter | D-Tube™ Dialyzer |
|--|---|---|--------------------------------|
| Faster optimization | ~20 minutes | <1 hour | 5 hours |
| Sensitive samples which may precipitate at higher concentrations | + | - | + |
| Post-dialysis concentration | + | + | - |
| Limited amounts of exchange solvent | + | + | - |
| Temperature sensitive | Minimal effect of cold temperature on speed | Minimal effect of cold temperature on speed | Cold temperature reduces speed |

Novel engineering provides unmatched buffer exchange.

The Amicon® Pro device is the first of its kind to offer dynamic, continuous buffer exchange by diafiltration. How does it work? The secret is in the design of the Amicon® Pro exchange device and tip. The lower portion of the exchange device is designed to exactly match the contours of the Amicon® Ultra-0.5 mL filter. The tip is tapered to maximize the external-to-internal volume ratio, ensuring that fresh buffer is slowly but consistently metered in, mixed with sample, and forced across the membrane and out. This delivers a continuous, controlled flow during desalting and buffer exchange, without multiple dilute-and-concentrate centrifugation steps. The results are the gentle recovery of greater than 95% of purified protein.

Fast: single spin
Gentle: unique design provides continuous diafiltration
Less Buffer: only 1.5 mL buffer required (given 0.5 mL initial sample)



The uniquely designed interface between the exchange tube tip and the Amicon® Ultra device enables greater than 99% buffer exchange in a single spin. Buffer exchange, as shown in this diagram, was measured by the replacement of a low-molecular weight dye (yellow) with clear buffer (black arrows); while a high-molecular weight dye (bright blue) was retained inside the Amicon® Ultra device.

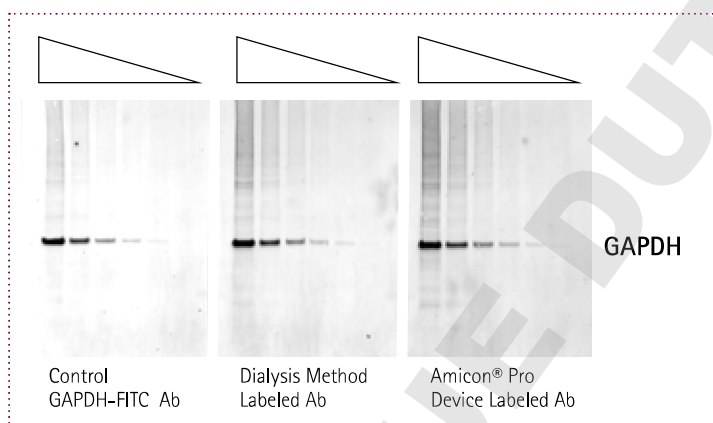
The gentleness of dialysis with the efficiency of diafiltration.

| | Dialysis cassette + concentrator | 0.5 mL diafiltration device (3 spin) | Amicon® Pro purification system |
|---|----------------------------------|--------------------------------------|---------------------------------|
| Process time | 16 hours | 50 min. | 20 min. |
| Recovery | 51% | > 95% | > 95% |
| Specific activity (signal/ µg GST-LPP) | 0.195 | 0.17 | 0.199 |

Gentler buffer exchange = greater activity. Eluted Samples of GST-lambda protein phosphatase (LPP) buffer exchanged and concentrated using Amicon® Pro device showed greater specific activity and percentage recovery than when prepared with a dialysis cassette (plus concentrator device) or 0.5 mL diafiltration spin column.

One hour antibody labeling.

The unique design of the exchange tip enables single spin diafiltration.



Generate FITC-labeled antibody in one hour. What's faster than labeling antibodies using other purification methods, and more economical than purchasing pre-labeled antibodies? Using Amicon® Pro purification systems for antibody labeling.

| Step | Dialysis-based buffer exchange pre/post labeling | Amicon® Pro purification system |
|---------------------------------------|--|---------------------------------|
| Buffer exchange | Overnight | 15 min |
| FITC labeling | 3 h | 30 min |
| Free FITC removal and buffer exchange | Overnight | 15 min |
| Total time | 3 days | 1 h |
| Antibody recovery | 39% | 72% |

Ordering Information

To choose the appropriate Amicon® Pro device, determine the molecular weight cut-off (MWCO) of your protein of interest and your desired affinity purification scheme.

| Amicon® Pro purification system – No Reagents Included | MWCO | | | | |
|---|-----------|-----------|-----------|-----------|-----------|
| | 3,000 | 10,000 | 30,000 | 50,000 | 100,000 |
| Amicon® Pro Purification System 12/pk | ACS500312 | ACS501012 | ACS503012 | ACS505012 | ACS510012 |
| Amicon® Pro Purification System 24/pk | ACS500324 | ACS501024 | ACS503024 | ACS505024 | ACS510024 |

What more will you discover using the Amicon® Pro system?

Find out at: www.merckmillipore.com/amiconpro

Featured Products

D-Tube™ Dialyzers

Fast and easy dialysis

Gently dialyze intractable or sensitive samples and prevent them from precipitation or over-concentration. Providing maximum efficiency, D-Tubes™ dialyzers are designed with a double membrane to spread the sample over a large surface area enabling complete dialysis in just two to five hours.



D-Tube™ Dialyzer Advantages:

> 89% Sample Recovery

- Low binding membrane and housing enhance sample recovery

Reliable and Easy to Use

- Secure design prevents sample loss due to leaks—no knots or clamps to loosen and leak
- Easy to open and close with a screw cap
- Rigid frame permits smooth sample withdrawal of submilliliter volumes—removing every last drop is easy

Convenient Sample Loading

- No need to use a syringe to load or remove samples. Simply load your sample with standard pipette tip
- Floating racks fit most standard beakers to hold devices in exchange buffer
- D-Tubes™ dialyzers can also be used to electroelute samples from agarose or acrylamide

Ordering Information

Available from www.merckmillipore.com/psp

| | | Product | D-Tube™ Mini | D-Tube™ Midi | D-Tube™ Maxi | D-Tube™ Mega | D-Tube™ Mega |
|-----------------------------------|---------------------------------|-------------------------------|--------------|--------------|----------------|--------------|--------------|
| Proteins/DNA/RNA/Oligonucleotides | Nominal Molecular Weight Cutoff | Maximum initial sample volume | 10 to 250 µL | 50 to 800 µL | 100 µL to 3 mL | 3 to 10 mL | 10 to 15 mL |
| MW | NMWCO | Qty/pk | | | | | |
| MW < 7 k | 3,500 | 10 | | 71506-3 | 71508-3 | 71739-3 | 71742-3 |
| | | 50 | | | | 71739-4 | 71742-4 |
| 7 < MW < 24 k | 7,000 | 10 | 71504-3 | 71507-3 | 71509-3 | 71740-3 | 71743-3 |
| | | 50 | | | | 71740-4 | 71743-4 |
| | | 1 plate of 96 | 71712-3 | | | | |
| 24 k < MW | 13,000 | 10 | 71505-3 | | 71510-3 | | |
| | | 50 | | | | | |
| | | 1 plate of 96 | 71713-3 | | | | |
| | Floating Rack | Product (Qty/pk) | Mini (10) | Midi (10) | Maxi (10) | Mega (10) | Mega (10) |
| | | | 71512-3 | 71513-3 | 71514-3 | 71748-3 | 71748-3 |

Fast and Easy Diafiltration With Amicon® Ultra Centrifugal Filters

Change buffers by gradually adding new solvent during simultaneous ultrafiltration

Because some macromolecules can lose activity or proper structure upon extreme changes of buffer conditions, use diafiltration, which involves removing microsolute by adding solvent to the sample being filtered at the same time that ultrafiltration is being applied.

Advantages of Amicon® Ultra diafiltration:

- Fast—buffer exchange in as few as two spins
- Efficient—requires minimal volume of exchange buffer, easily contained in reservoir
- Easy to use—simply load your sample with standard pipette tip
- Enables simultaneous concentrating and desalting

For product selection, consult the Amicon® Ultra selection chart on pages 32–34.



Centrifugal Concentration Devices

Featured Products

Amicon® Ultra Centrifugal Filters

Fast and easy protein concentration

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.

Amicon® Ultra Centrifugal Filter Advantages:

Maximize Concentration with Highest Protein Recovery

True Engineered Dead Stop

- Avoids spinning to dryness
- Provides a predictable concentration factor
- No need to calibrate for several samples to run in parallel

Reverse Spin Recovery

- Reverse spin devices enable you to maximize protein recovery, especially with small dilute samples, without introducing pipetting errors
- Low binding membrane and polypropylene housing for >90% sample recovery

Fast and Efficient Concentration Without Compromise

Ultracef® Low-binding Membranes

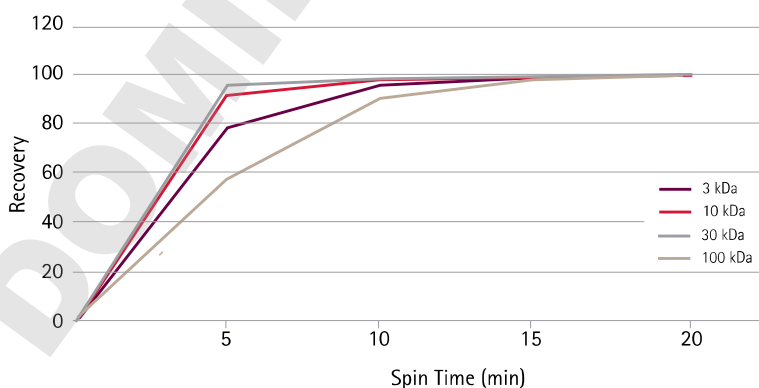
- Vertical membrane design aligns with filtrate rather than perpendicular for less clogging, less waste and faster filtration
- Ultra-fast sample processing achieving concentration in as little as 10 minutes
- 25- to 80-fold concentration in a single step

Broad Chemical Compatibility

- Heat-sealed membrane eliminates adhesives and downstream extractables
- Large spectrum of compatibility
- Compatible with pH 1 to 9

Reliable Samples

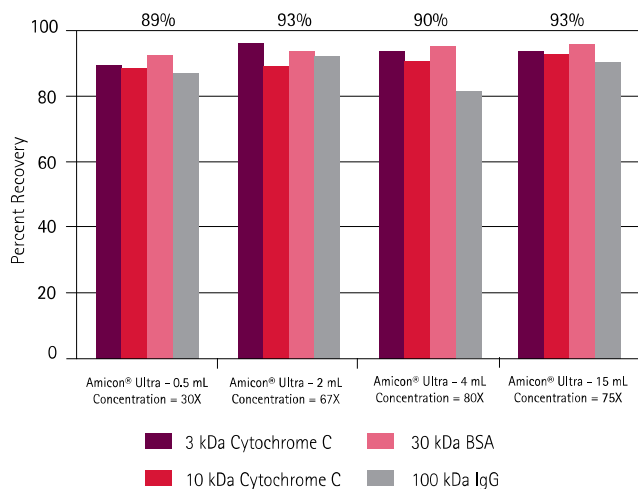
- Spin precious samples with confidence in one robust, sleek unit that prevents leakage



Amicon® Ultra 4 mL Filters—Fast Spin Times with Excellent Recovery

Average spin time for Amicon® Ultra-4 mL Filters:

Four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, 30 kDa BSA, and 100 kDa IgG) were tested on the Amicon® Ultra-4mL Filters for percent recovery and spin time. The data show that greater than 95% of all protein was recovered in 15 minutes or less.



Consistently high recovery of diverse proteins with Amicon® Ultra filters

Concentration and percent recovery using Amicon® Ultra Filters: 4 different devices (Amicon® Ultra-0.5 mL, Amicon® Ultra-2 mL, Amicon® Ultra-4 mL, Amicon® Ultra-15 mL), were tested with four different proteins (3 kDa Cytochrome C, 10 kDa Cytochrome C, 30 kDa BSA and 100 kDa IgG) to determine percent recovery and concentration factor.

To select an Amicon® Ultra Centrifugal Filter, identify the starting volume, molecular weight of protein or nucleic acid being concentrated, final volume and concentration factor.

Then consult the product selection chart below to choose the Amicon® Ultra filter with the right molecular weight cutoff (MWCO).



| Starting Volume | < 0.5 mL | < 2 mL | < 4 mL | < 15 mL |
|-----------------|----------|--------|--------|---------|
|-----------------|----------|--------|--------|---------|

Proteins

| Molecular Weight (MW) | < 0.5 mL | < 2 mL | < 4 mL | < 15 mL |
|-----------------------|----------|---------|---------|---------|
| 6 < MW < 20 k | 3,000 | 3,000 | 3,000 | 3,000 |
| 20 < MW < 60 k | 10,000 | 10,000 | 10,000 | 10,000 |
| 60 < MW < 100 k | 30,000 | 30,000 | 30,000 | 30,000 |
| 100 < MW < 200 k | 50,000 | 50,000 | 50,000 | 50,000 |
| 200 k < MW | 100,000 | 100,000 | 100,000 | 100,000 |

Single-Stranded and Double-Stranded Nucleic Acids

| Length | < 0.5 mL | < 2 mL | < 4 mL | < 15 mL |
|-------------|----------|--------|--------|---------|
| 137-1159 bp | 30,000 | 30,000 | 30,000 | 30,000 |

Nanoparticles

| Particle Diameter (DIA) | < 0.5 mL | < 2 mL | < 4 mL | < 15 mL |
|-------------------------|----------|---------|---------|---------|
| 1.5 < dia < 3 nm | 3,000 | 3,000 | 3,000 | 3,000 |
| 3 < dia < 5 nm | 10,000 | 10,000 | 10,000 | 10,000 |
| 5 < dia < 7 nm | 30,000 | 30,000 | 30,000 | 30,000 |
| 7 < dia < 10 nm | 50,000 | 50,000 | 50,000 | 50,000 |
| 10 nm < dia | 100,000 | 100,000 | 100,000 | 100,000 |

MWCO: Molecular Weight Cut Off

10,000 MWCO Amicon® Ultra-4 and -15 filters are both CE marked for *in vitro* diagnostic use.

Once you've chosen the right Amicon® Ultra filter for your needs, choose your rotor, G force and spinning time for concentrating your molecule.

Designed as standard 1.5 mL, 15 mL conical or 50 mL conical tubes, Amicon® Ultra filters fit all standard rotor types.



CHOOSE A ROTOR
AND G FORCE

| | | | | |
|--------------------------|----------------------------------|---------------------------------|---|----------------|
| Starting Volume | < 0.5 mL | < 2 mL | < 4 mL | < 15 mL |
| Final Volume | 15–20 µL | 15–70 µL | 50 µL | 200 µL |
| Design of the Device | 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 100,000 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 |

CONCENTRATION
FACTOR

| | | | | |
|----------------------|----------------------------|----------------------------|-------|--------|
| Final Volume | 15–20 µL with reverse spin | 15–70 µL with reverse spin | 50 µL | 200 µL |
| Concentration Factor | X25–X30 | X14–X67 | X80 | X75 |

ADJUST
SPINNING TIME

For Proteins and Nanoparticles

| | | | | |
|---------|---------|---------|---------|---------|
| 3,000 | 30 min. | 60 min. | 40 min. | 40 min. |
| 10,000 | 15 min. | 40 min. | 15 min. | 20 min. |
| 30,000 | 10 min. | 20 min. | 10 min. | 20 min. |
| 50,000 | 10 min. | 15 min. | 10 min. | 15 min. |
| 100,000 | 10 min. | 30 min. | 10 min. | 15 min. |

Single-Stranded and Double-Stranded Nucleic Acids

| | | | | |
|--------|---------|---|----------------------------------|----------------------------------|
| 30,000 | 10 min. | 15 min., fixed angle 40 min., swinging rotor | 10 min., 5,000 g, fixed angle | 10 min., 5,000 g, fixed angle |
|--------|---------|---|----------------------------------|----------------------------------|

Visit www.merckmillipore.com/psp to check both chemical compatibility and centrifuge/rotor compatibility of Amicon® Ultra devices.

Amicon® Ultra Centrifugal Filters

| | Product | Amicon® Ultra-0.5 | Amicon® Ultra-2 | Amicon® Ultra-4 | Amicon® Ultra-15 |
|--------------|---|----------------------|--------------------|--------------------|---------------------|
| | Maximum initial sample volume (mL) | 0.5 | 2 | 4 | 15 |
| | Final concentrate (retentate) volume (µL) | 15–20 | 15–70 | 30–70 | 150–300 |
| MWCO | Qty/Pk | | | | |
| 3,000 MWCO | 8 | UFC500308 | | UFC800308 | UFC900308 |
| | 24 | UFC500324 | UFC200324 | UFC800324 | UFC900324 |
| | 96 | UFC500396 | | UFC800396 | UFC900396 |
| | 500 | UFC5003BK | | | |
| 10,000 MWCO | 8 | UFC501008 | | UFC801008* | UFC901008* |
| | 24 | UFC501024 | UFC201024 | UFC801024* | UFC901024* |
| | 96 | UFC501096 | | UFC801096* | UFC901096* |
| | 500 | UFC5010BK | | | |
| 30,000 MWCO | 8 | UFC503008 | | UFC803008 | UFC903008 |
| | 24 | UFC503024 | UFC203024 | UFC803024 | UFC903024 |
| | 96 | UFC503096 | | UFC803096 | UFC903096 |
| | 500 | UFC5030BK | | | |
| 50,000 MWCO | 8 | UFC505008 | | UFC805008 | UFC905008 |
| | 24 | UFC505024 | UFC205024 | UFC805024 | UFC905024 |
| | 96 | UFC505096 | | UFC805096 | UFC905096 |
| | 500 | UFC5050BK | | | |
| 100,000 MWCO | 8 | UFC510008 | | UFC810008 | UFC910008 |
| | 24 | UFC510024 | UFC210024 | UFC810024 | UFC910024 |
| | 96 | UFC510096 | | UFC810096 | UFC910096 |
| | 500 | UFC5100BK | | | |

*Certified for clinical applications.

To use the online Amicon® selector tool to choose the perfect filter and view protocols visit: www.merckmillipore.com/AmiconSelect

Specialized Concentration Devices

Concentration of gDNA and Protein

Microcon® DNA Fast Flow Filter

Optimized for the concentration and recovery of genomic DNA with SDS buffer. The low nonspecific binding characteristics of the membrane and the other device components, coupled with its medical-grade o-ring seal, allows the device to accommodate several wash steps with minimal sample loss.

Microcon® DNA Fast Flow Advantages:

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

Microcon® Centrifugal Filters

Simply and efficiently concentrate and desalt solutions of any macromolecule with the low-binding Ultracel® membrane, using any centrifuge that can accept 1.5 mL tubes.

Microcon® Advantages:

- Dual-cycle EtO treatment on the Microcon® PCR Grade Filter has been shown to render contaminating DNA unamplifiable
- 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 tube
- Concentration factors up to 100X



Application Guidelines

| Application | Microcon® Device | | |
|---|------------------|-----|---------------|
| | 10K | 30K | DNA Fast Flow |
| Peptide and growth factor concentration | • | | |
| Protein concentration and desalting of columns eluates | • | • | |
| Protein concentration before electrophoresis or other assays | • | • | |
| Protein removal prior to HPLC | • | • | |
| Purification of macromolecular components found in tissue culture extracts and cell lysates | • | • | |
| Concentration of biological samples (antigens, antibodies, enzymes) | | • | |
| Concentration of gDNA with or without SDS buffer | | • | • |
| Concentration and desalting of nucleic acids (single-or double-stranded) | • | • | • |
| Removal of labeled nucleotides | • | • | • |
| Removal of labeled amino acids | • | • | • |
| Removal of primers from amplified DNA | | • | • |
| Removal of linkers prior to cloning | | • | • |

Ordering Information

| MWCO | Qty/Pk | Catalog No. | Description | Volume, mL | Min. final concentrate volume, µL |
|------|--------|-------------|--|------------|-----------------------------------|
| 10 | 100 | MRCPR010 | Microcon® filter, Ultracel®-10 membrane, 10kDa | 0.5 | 5-50 |
| 30 | 100 | MRCF0R030 | Microcon® filter, Ultracel®-30 membrane, 30kDa | 0.5 | 5-50 |
| - | 100 | MRCF0R100 | Microcon® filter, Ultracel® DNA Fast Flow Membrane | 0.5 | 5-50 |
| -- | 20 | MRCF0R100ET | Microcon® DNA Fast Flow PCR Grade Filter | 0.5 | 5-50 |

Spin filters for clarification, filtration, and sterilization

Ultrafree®-MC and Ultrafree®-CL centrifugal filters remove particles and precipitates from aqueous and some solvent based samples. These fast filtration units provide highly reproducible performance for sample recovery. Ultrafree® centrifugal filters are ideal for use in protein and nucleic acid solutions.

Ultrafree®-MC filter advantages:

- Five different pore sizes from 0.1 to 5.0 µm
- Pre-sterilized units also available
- Fast filtration and highly reproducible performance
- Use in fixed-angle rotors for 1.5 mL tubes

Ultrafree®-CL filter advantages:

- High recovery Durapore® (PVDF) and hydrophilic PTFE membranes
- Five different pore sizes from 0.1 to 5.0 µm
- Pre-sterilized units also available
- Fast filtration and highly reproducible performance
- Use in fixed-angle rotors for 15 mL tubes

Sterile Ultrafree®-MC and CL centrifugal filter units with microporous membrane

- Easy, pre-sterilized, centrifugal sample clarification units for either 0.5 mL (MC) or 2 mL (CL) maximum volumes
- High recovery Durapore® (PVDF) membrane
- Fast filtration and highly reproducible performance
- Use in fixed-angle rotors for 1.5 mL tubes (MC) or 15 mL tubes (CL)



| | Pore Size (µm) | Color | Sterility | Qty/Pk | Catalog No. | |
|--|--|-------------|-------------|-------------|-------------|-----------|
| Filter Units with Microporous Durapore® PVDF Membrane | | | | | | |
| Ultrafree®-MC Filter | 0.1 | Orange | Non-sterile | 25 | UFC30VV25 | |
| | | | | 100 | UFC30VV00 | |
| | | | | 250 | UFC30GVNB | |
| | 0.22 | Yellow | Non-sterile | 25 | UFC30GV25 | |
| | | | | 100 | UFC30GV00 | |
| | | | | 250 | UFC30GVNB | |
| | 0.45 | Red | Non-sterile | 50 (5x10) | UFC30GV0S | |
| | | | | 25 | UFC30HV25 | |
| | | | | 100 | UFC30HV00 | |
| | 0.65 | Purple | Non-sterile | 25 | UFC30DV25 | |
| | | | | 100 | UFC30DV00 | |
| | | | | 50 (5x10) | UFC30DV0S | |
| 6 | Dark Green | Non-sterile | 100 | UFC30SV00 | | |
| Ultrafree®-CL Filter | 0.1 | Orange | Non Sterile | 25 | UFC40VV25 | |
| | | | | 100 | UFC40VV00 | |
| | | | | 250 | UFC40GV00 | |
| | 0.22 | Yellow | Non Sterile | 25 | UFC40GV25 | |
| | | | | 100 | UFC40GV00 | |
| | | | | 50 (5x10) | UFC40GV0S | |
| | 0.45 | Red | Non Sterile | 25 | UFC40HV25 | |
| | | | | 100 | UFC40HV00 | |
| | | | | 25 | UFC40DV25 | |
| | 5 | Dark Green | UFC40SV25 | 25 | UFC40SV25 | |
| | 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 | |

Concentrate high solute samples

Centriprep® centrifugal filters are disposable ultrafiltration devices used for purifying, concentrating, and desalting biological samples (2–15 mL volume range) and for filtration applications. Offering a high flow rate, these filters come complete and are easy to use with a twist-lock cap, a filtrate collector containing a low adsorptive Ultracel® regenerated cellulose membrane, plus an air-seal cap for sample isolation.

Centriprep® filter advantages and applications:

- Unique inverse flow mode of operation with large deadstop
- Concentrate and purify particle-laden solutions of high concentrations with Ultracel® membrane
- Fast sample processing
- Fits standard swinging-bucket rotor for 50 mL tubes
- Concentrate and purify particle-laden solutions or high concentrations
- Separate low MW solutes from fermentation broths, cell culture media, cell lysates



Ordering Information

| MWCO | Qty/Pk | Catalog No. | Description | Volume, mL | Min. final concentrate volume, µL |
|------|--------|-------------|---------------------------------|------------|-----------------------------------|
| 3 | 24 | 4302 | Centriprep® YM-3, 3 kDa NMWL | 15 | 700 |
| 3 | 96 | 4303 | Centriprep® YM-3, 3 kDa NMWL | 15 | 700 |
| 10 | 24 | 4304 | Centriprep® YM-10, 10 kDa NMWL* | 15 | 700 |
| 10 | 96 | 4305 | Centriprep® YM-10, 10 kDa NMWL* | 15 | 700 |
| 30 | 24 | 4306 | Centriprep® YM-30, 30 kDa NMWL* | 15 | 700 |
| 30 | 96 | 4307 | Centriprep® YM-30, 30 kDa NMWL* | 15 | 700 |
| 50 | 24 | 4310 | Centriprep® YM-50, 50 kDa NMWL | 15 | 700 |
| 50 | 96 | 4311 | Centriprep® YM-50, 50 kDa NMWL | 15 | 700 |

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

Clinical Ultrafiltration

Separate free from protein-bound solute

The Centrifree® filter was designed with the clinical laboratory in mind, these devices rapidly and efficiently separate free from protein-bound microsolutes in small volumes (0.15–1.0 mL) of serum, plasma, and other biological samples using ultrafiltration. Accurate partitioning occurs in minutes without dilution, change in physiologic pH, ion composition, or unbound microsolutes concentration. These devices contain low-adsorptive hydrophilic membranes and O-rings without plasticizers to ensure excellent recovery.

Centrifree® filter advantages and applications:

- Separation of free from bound microsolutes in serum, plasma, and other biological samples
- Determine free therapeutic drugs, testosterone, thyroxine
- Binding studies
- New drug investigations
- Deproteinization

Ordering Information

| MWCO | Qty/Pk | Catalog No. | Description | Volume, mL | Min. final concentrate volume, µL |
|------|--------|-------------|---|------------|-----------------------------------|
| 10 | 50 | 4104 | Centrifree® Ultrafiltration device with Ultracel® YM-T membrane | 1 | 50 |



Concentrate Multiple Clinical Samples

Minicon® concentrators are non-sterile, disposable, multiwell ultrafiltration devices designed for concentrating macromolecules in clinical specimens such as urine, cerebrospinal fluid (CSF) or other biological solutions. The concentrators, which require no additional equipment and can be operated unattended, are used by researchers and clinical laboratories worldwide as a preparatory step to increase the sensitivity of subsequent tests.



Minicon® concentrator advantages and applications:

- Concentrate urine and cerebrospinal fluid to intensify proteins that indicate abnormal or pathological states prior to analysis by electrophoresis or immunoelectrophoresis (e.g., Bence Jones proteins in urine)
- Static concentrator, requiring no accessories
- Absorbent pulls solvent and salts through ultrafilter, concentrating sample

Ordering Information

| MWCO | Qty/Pk | Catalog No. | Description | Volume, mL | Min. final concentrate volume, μ L |
|------|--------|-------------|------------------------------|------------|--|
| 15 | 40 | 9031 | Minicon® B15, 8 cells/unit | 5 | 50 |
| 15 | 50 | 9051 | Minicon® CS15, 10 cells/unit | 2.5 | 30 |
| 15 | 50 | 9051 | Minicon® CS15, 10 cells/unit | 2.5 | 30 |

Large Volume Concentration

Convenient alternative to stirred cells

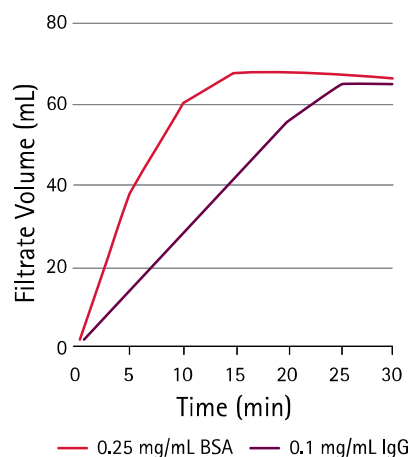
The Centricon® Plus-70 centrifugal filter is designed for rapid processing of aqueous biological solutions in volumes ranging from 15 to 70 mL. Centricon® filters concentrate most 70 mL solutions down to 350 µL in as little as 25 minutes. Samples are typically concentrated in the 50X to 200X range, depending on the sample type and starting sample volume. These units are a convenient alternative to dialysis, lyophilization, precipitation techniques or stirred cells.

Centricon® Plus-70 advantages and applications

- >90% typical recovery
- Low hold-up volume
- Polypropylene housing minimizes binding
- True dead stop prevents spinning to dryness
- Concentrating and desalting chromatography column eluates
- Concentrating monoclonal antibodies
- Concentrating proteins or viruses from culture supernatants
- Clarifying tissue homogenates and cell lysates

Performance

Spin time with respect to filtrate volume



Ordering Information

| MWCO | Qty/Pk | Catalog No. | Description | Volume, mL | Min. final concentrate volume, µL |
|------|--------|-------------|-------------------------|------------|-----------------------------------|
| 3 | 8 | UFC700308 | Centricon® Plus-70 3K | 70 | 350 |
| 10 | 8 | UFC701008 | Centricon® Plus-70 10K | 70 | 350 |
| 30 | 8 | UFC703008 | Centricon® Plus-70 30K | 70 | 350 |
| 100 | 8 | UFC710008 | Centricon® Plus-70 100K | 70 | 330 |

Stirred Cells: 3 mL to 400 mL concentration

Amicon® stirred cells provide high flow rates with solutions up to 10% macrosolute concentration and are capable of rapid concentration, or salt removal followed by concentration in the same unit. For protein concentration, gas pressure is applied directly to ultrafiltration cell. Solutes above the membrane's molecular weight (MW) cut-off are retained in cell, while water and solutes below the cut-off pass into the filtrate and out of cell.

Advantages

- Gentle magnetic stirring minimizes concentration polarization and shear denaturation.
- All stirred cells can be autoclaved.
- Five different sizes to handle volumes from 3 mL to 400 mL
- High flow rates with solutions up to 10% macrosolute concentration

Applications

- Concentrate, diafilter, and exchange buffers for macromolecule solutions including proteins, enzymes, antibodies and viruses.



Available in five sizes

| Max. Working Volume | Catalog No. |
|---------------------|-------------|
| 3 mL | 5125* |
| 10 mL | 5121* |
| 50 mL | UFSC05001 |
| 200 mL | UFSC20001 |
| 400 mL | UFSC40001 |

*Note: The 3 mL and 10 mL stirred cells are available in the Series 8000 model only.

Introducing the new Amicon® Stirred Cell.

Order the new 50 mL, 200 mL, or 400 mL stirred cells and you will experience the same performance to which you're accustomed: gentle, high recovery of macrosolutes, thorough buffer exchange, membrane flexibility and ability to monitor filtration progress. In addition, you will enjoy many workflow-enhancing features.

What's new about the updated stirred cells:

- Ergonomic benefits: you will love how easy it is to open, close and assemble the new Amicon® stirred cell!
- Quick connectors to tubing for easy, secure setup.
- Integrated safety features: with screw threads and a pressure relief valve, there's no need for external housing. This means easier assembly and disassembly, and very clear confirmation that the device is properly assembled.
- Overall superior integrity (no leaking).
- Broader selection of membrane discs.
- Fully revised user guide with clearer instructions for operation and how to connect to your gas source.
- Better spare part and accessory support.
- More secure stir bar eliminates risk of damage to your membrane.

Ultracel®, Biomax®, and Durapore® Membrane Discs for Use in Stirred Cells

To concentrate or desalt dilute solutions, use Ultracel® regenerated cellulose membranes. The hydrophilic, tight microstructure of Ultracel® membranes assures the highest possible retention with the lowest possible adsorption of protein, DNA or other macromolecules.

- Membranes available in 1, 3, 5, 10, 30 and 100 kDa nominal molecular weight limit (NMWL).
- Filter diameters available in 25, 44.5, 47, 63.5, 76, 90 and 150 mm.

To concentrate or desalt higher volumes of more concentrated samples (recommended for protein concentrations greater than 1.0 µg/mL), use Biomax® polyethersulfone (PES) membranes. These membranes are recommended for samples such as serum, plasma, or conditioned tissue culture media.

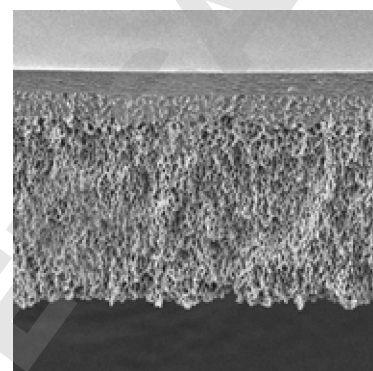
- Membranes available in 5, 10, 30, 50, 100, 300, and 500 kDa nominal molecular weight limit (NMWL).
- Filter diameters available in 25, 44.5, 47, 63.5, 76, 90 and 150 mm.

For ordering information, please visit www.merckmillipore.com/ufdiscs

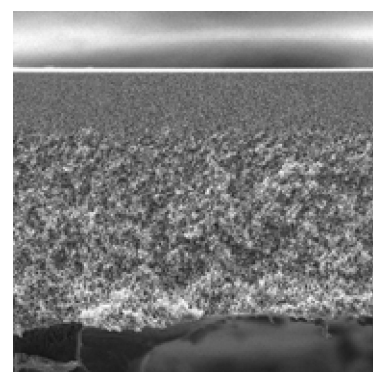
For large-volume microfiltration, choose Durapore® PVDF membrane discs for your stirred cell.

- Membranes available in 0.1, 0.2 and 0.45 µm pore sizes
- Filter diameters available in 63.5 and 70 mm.

Contact customer service for Durapore® discs ordering information.



Ultracel® regenerated cellulose ultrafiltration membrane.



Biomax® polyethersulfone ultrafiltration membrane.

Stirred Cell Accessories Expand Your Capabilities.

Amicon® Stirred Cell Selector Valve

Valve with sliding control for instant switching from concentration to diafiltration, or switching gas and liquid lines simultaneously. Simplifies operation and avoids the need for multiple T-fittings and valves.

Amicon® Stirred Cell Manifold

For instant direction of gas pressure or liquid flow in multi-cell or multi-reservoir systems. Can pressurize up to 3 cells or reservoirs from one gas source or feed several cells from one reservoir.

Amicon® Stirred Cell Reservoir

This 800 mL auxiliary reservoir increases the volume capacity of stirred cells. When pressurized from an external gas source, it automatically replenishes liquid in the cell's built-in reservoir during filtration. The reservoir may also be used to store dialysate during diafiltration or dialysis.

Goodbye, Bradford Assays! Drive your research forward with IR-based quantitation.

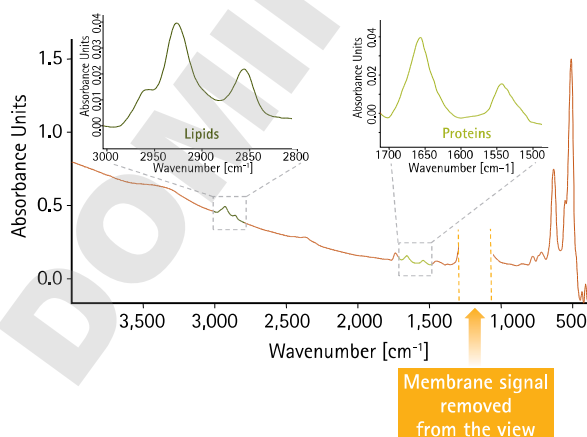
Did you know that one of the most accurate, universal methods for quantifying total protein is by using infrared (IR)-based protein quantitation?

That's why Merck Millipore developed the Direct Detect® spectrometer, to make the power and accuracy of IR spectroscopy accessible to any life science laboratory.

Now you can achieve truly accurate results without the pitfalls of colorimetric assays, even for many lysates and complex samples.

Quit Assays Forever—Quantitate Directly.

Learn more at: www.merckmillipore.com/DirectDetect



Accurate IR-based protein quantitation in a lipid-rich lysate. The most intense regions of lipid absorbance are distinct from the protein's Amide I signal.



www.merckmillipore.com

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Germany: 069 86798021

Italy: 848 845 645

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Switzerland: 0848 645 645

United Kingdom: 0870 900 4645

For other countries across Europe,
please call: +44 (0) 115 943 0840

Or visit: www.merckmillipore.com/offices

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