

MILLIPORE
SIGMA

REFINE PROTEIN PREPARATION.

Tools for better protein analysis.



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INTRODUCTION

Today, researchers are challenged to create high quality samples for meaningful protein analysis, often using cumbersome traditional sample preparation methods. With over 50 years of experience in developing protein sample preparation technologies, MilliporeSigma 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 us to support your research with maximum yields of intact, functional proteins.

KEY FEATURES

Unmatched Flexibility

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

Diverse downstream applications

Our reagents 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.

TABLE OF CONTENTS

Protein Extraction	4
Protein Extraction with Cell Lysis Reagents (“Busters”)	5
Fast, simple, gentle protein extraction from <i>E. coli</i> , yeast, insect and mammalian cells	
Cell Lysis and Nucleic Acid Removal Enhancers	6
Increase protein extraction yield and purity with Benzonase® and rLysozyme™ reagents	
Protein Extraction with ProteoExtract® Kits	8
Extract proteins from different fractions of the mammalian cell, including membrane, nucleus, cytosol and cytoskeleton	
Protein Extraction with Inhibitors	9
Protease and phosphatase inhibitor cocktails to prevent proteolysis and dephosphorylation of proteins	
Protein Purification and Depletion	11
Affinity Purification with PureProteome™ Magnetic Beads	12
Ideal for small volume applications such as immunoprecipitation and recombinant protein screening	
Affinity Purification with Recombinant Fusion Tags	16
Protein Depletion	19
Protein depletion reagents: Seppro® Protein Depletion, PureProteome™ reagents, ProteoExtract® depletion, ProteoPrep® products	
Amicon® Pro Purification System	20
Purify, exchange buffer and/or concentrate in a single device	
Protease Cleavage Enzymes	22
Recombinant enterokinase, factor Xa, HRV 3C protease, thrombin and other enzymes for cleaving fusion proteins	
Protein Buffer Optimization and Sample Concentration	24
Dialysis and Buffer Exchange Devices	25
Amicon® Pro Purification System, D-Tube™ Dialyzers and Amicon® Ultra centrifugal filters for protein sample diafiltration and buffer exchange	
Centrifugal Concentration Devices	29
Amicon® Ultra filters for fast and effective protein concentration	
Specialized Concentration Devices	33
Microcon® and Ultrafree® filters for efficient purification, concentration, and desalting of biological samples	
Clinical Filtration Devices	36
Centrifree® concentrators for concentration or partition of body fluids or other biological specimens	
Large Volume Concentration Devices	37
Centricon® Plus-70 centrifugal filters 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.

Our 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	
<i>E. coli</i>						
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™ 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 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”)

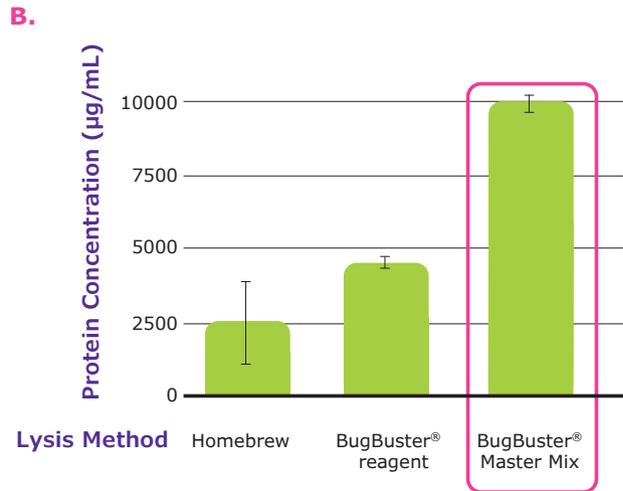
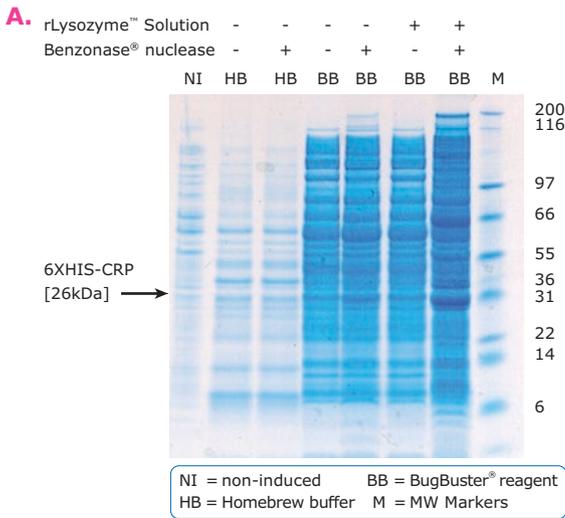
Featured Products

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 produced lysates with the highest 6XHIS-CRP yields.

(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.

How do I choose between BugBuster® Products?

Components of Bacterial Lysis Reagents

	BugBuster® Reagent	Buffer	Benzonase® Nuclease	rLysozyme™ Solution	Notes
BugBuster® Reagent	■	■			
BugBuster® 10X reagent	■				Flexibility to customize dilution and buffer composition
BugBuster® Plus Benzonase® Nuclease	■	■	■		2 separate vials for greater flexibility
BugBuster® Plus Lysonase™ Kit	■	■	■	■	2 separate vials for greater flexibility
BugBuster® Master Mix	■	■	■	■	1 convenient reagent
PopCulture® Reagent	■	■			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 cells that preserves the phosphorylation state of sample proteins.

YeastBuster™ reagent — Extract proteins from yeast and plants without mechanical disruption or 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 extraction from total insect cell culture (in suspension or adherent) without the need for centrifugation.

Ordering Information

Application	Description	Fisher Scientific Cat. No.
Bacteria	BugBuster® Protein Extraction Reagent	70-584-1000ML
	BugBuster® Master Mix	71-456-4
	BugBuster® Plus Benzonase® Nuclease	70-750-3
	BugBuster® Plus Lysonase™ Kit	71-370-3
	BugBuster® 10X Protein Extraction Reagent	70-921-3
	PopCulture® Reagent	71-092-3
Mammalian	CytoBuster™ Protein Extraction Reagent	71-009-3MI
	NucBuster™ Protein Extraction Reagent	71-183-3
	PhosphoSafe™ Extraction Reagent	71-296-4
Yeast	YeastBuster™ Protein Extraction Reagent	71-186-4
Insect	Insect PopCulture® Reagent	71-187-3

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 protein yields.

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.

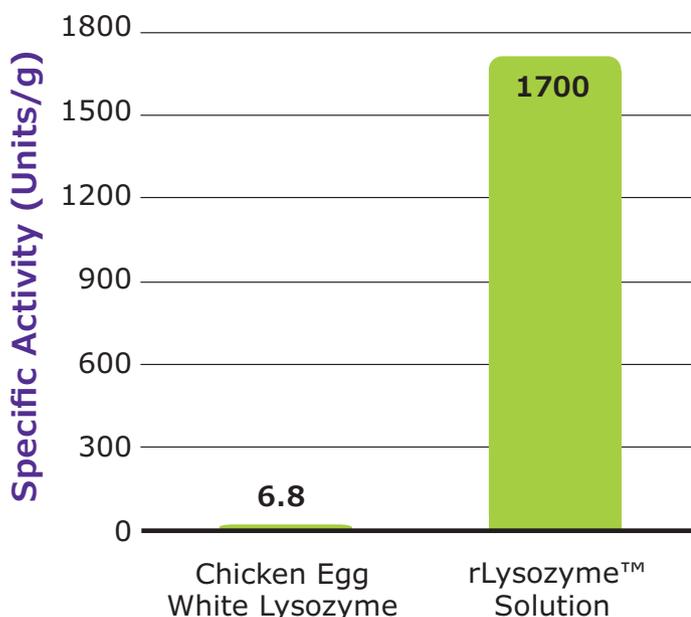


***E. coli* lysate without Benzonase® Nuclease.**
 Gooney, viscous, difficult to handle.

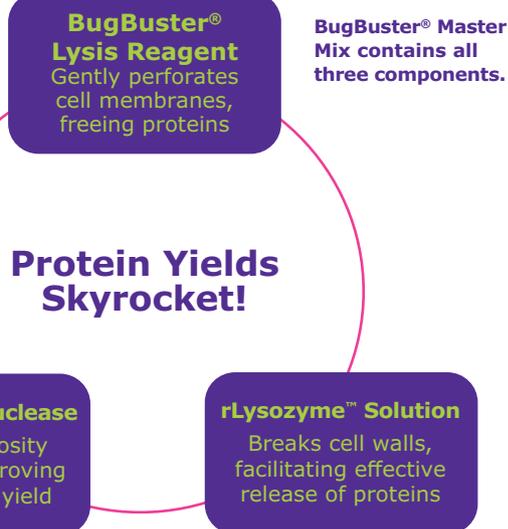
rLysozyme™ Solution

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™ solution (1700 KU/mg) for *E. coli* lysis is 250 times greater than that of traditional chicken egg white lysozyme. rLysozyme™ solution is optimally active at physiological pH. Very small amounts of rLysozyme™ solution 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™ solution exhibits 250 times higher specific activity than chicken egg white activity when measured using a standard activity assay.



Ordering Information

Description	Fisher Scientific Cat. No.
Benzonase® Nuclease, Purity > 90 %	70-746-4
Benzonase® Nuclease HC, Purity > 90 %	71-205-3
Benzonase® Nuclease, Purity > 99 %	70-664-3
Benzonase® Nuclease HC, Purity > 99 %	71-206-3
rLysozyme™ Solution	71-110-3
Chicken Egg White Lysozyme Solution	71-412-3
Lysonase™ Bioprocessing Reagent	71-230-3

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.

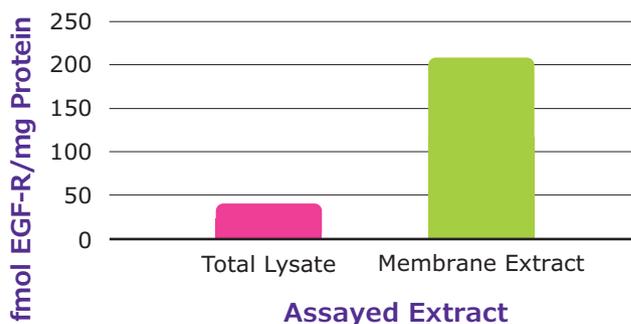
Applications of S-PEK:

- 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 fluorescently-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.

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



Notably 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 an 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

Application	Description	Fisher Scientific Catalog No.
Organelle Fractionation	ProteoExtract® Subcellular Protein Extraction Kit	53-979-01KIT
	ProteoExtract® Complete Mammalian Protein Extraction Kit	53-977-91KIT
	ProteoExtract® Cytosol/Mitochondria Fractionation Kit	QIA881KIT
	ProteoExtract® Native Cytoskeleton Enrichment Kit	17-102-10
	ProteoExtract® Cytoskeleton Enrichment and Isolation Kit	17-101-95
Membrane Proteins	ProteoExtract® Native Membrane Protein Extraction Kit	44-481-01KIT
	ProteoExtract® Transmembrane Protein Extraction Kit	71-772-3
Mass Spec Peptide Enrichment	ProteoExtract® All-in-One Trypsin Digestion Kit	65-021-21KIT
	ProteoExtract® Glycopeptide Enrichment Kit	72-103-3
	ProteoExtract® Phosphopeptide Enrichment TiO ₂ Kit	53-972-21KIT

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:

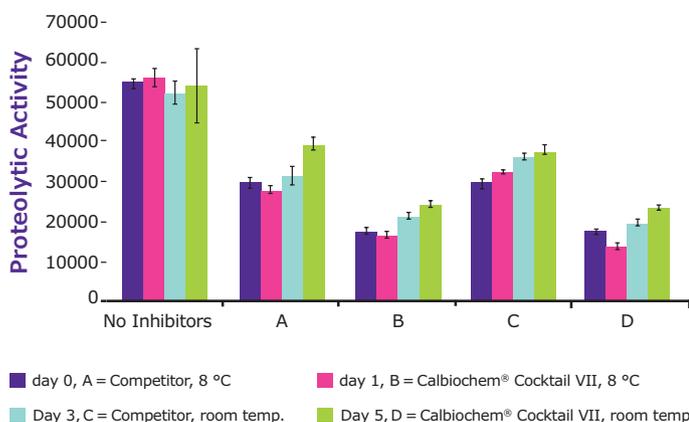
- Convenient—Flexible protocol and ready-to-use formulations
- Consistent—High quality ensures reproducibility and excellent inhibition over a wide range of protease classes
- Flexible—Comprehensive selection of specific cocktail formulations designed to inhibit proteolytic activity from most tissue 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

Featured Protease Inhibitor Cocktails

Protease Inhibitor Cocktail Set III, EDTA-Free (Fisher Scientific Cat. No. 53-913-41SET)

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.

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. The ability of the inhibitors to inhibit the proteolytic activity of PRONASE® reagent (Fisher Scientific Cat. No. 53-708-8100KU) 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, 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.

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. MilliporeSigma 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 (Fisher Scientific Cat. No. 52-462-51SET)

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.fishersci.com

Description	Recommended Application	Fisher Scientific Cat. No.
Protease Inhibitor Cocktail Set I	General Use	53-913-11VL
Protease Inhibitor Cocktail Set II	Bacterial cell extracts (except those intended for metal chelation chromatography)	53-913-21SET
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	53-913-41SET
Protease Inhibitor Cocktail Set IV	Fungal and yeast cell extracts	53-913-61SET
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	53-913-710VL
Protease Inhibitor Cocktail Set VI	Plant cell extracts	53-913-31SET
Protease Inhibitor Cocktail Set VII	Proteins containing His•Tag® sequences	53-913-81SET
Serine Protease Inhibitor Cocktail	Broad range serine protease inhibition	56-500-01VL
Phosphatase Inhibitor Cocktail Set I	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A	52-462-41SET
Phosphatase Inhibitor Cocktail Set II	Protection against acid and alkaline phosphatases and Protein Tyrosine Phosphatases (PTPs)	52-462-51SET
Phosphatase Inhibitor Cocktail Set III	Protection against acid, alkaline and Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)	52-462-71SET
Phosphatase Inhibitor Cocktail Set IV	Protection against alkaline phosphatases and Ser/Thr phosphatases such as PP1 and PP2A	52-462-81SET
PhosphoSafe™ Extraction Reagent	Protection against Ser/Thr phosphatases and Protein Tyrosine Phosphatases (PTPs)	71-296-3

PROTEIN PURIFICATION AND DEPLETION

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. We offer a wide range of tools for protein purification, including affinity magnetic beads, affinity agarose resins, the Amicon® Pro purification system and protease cleavage enzymes. To ensure that samples are enriched for protein(s) of interest, our depletion reagents eliminate common irrelevant, abundant proteins that may confound protein analysis.



- 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.



Affinity Purification with PureProteome™ Magnetic Beads

PureProteome™ Protein A and G Beads: Fast and easy immunoprecipitation

Traditional methods require hours of incubation time and 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.

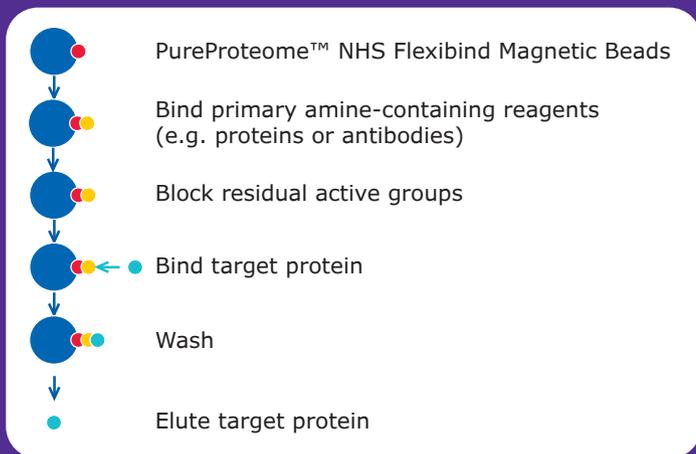
Advantages of PureProteome™ Immunoprecipitation:

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

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. Customization of beads requires only that the target ligand has a free amine group.



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

- **Fast:** Customize your own beads 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)

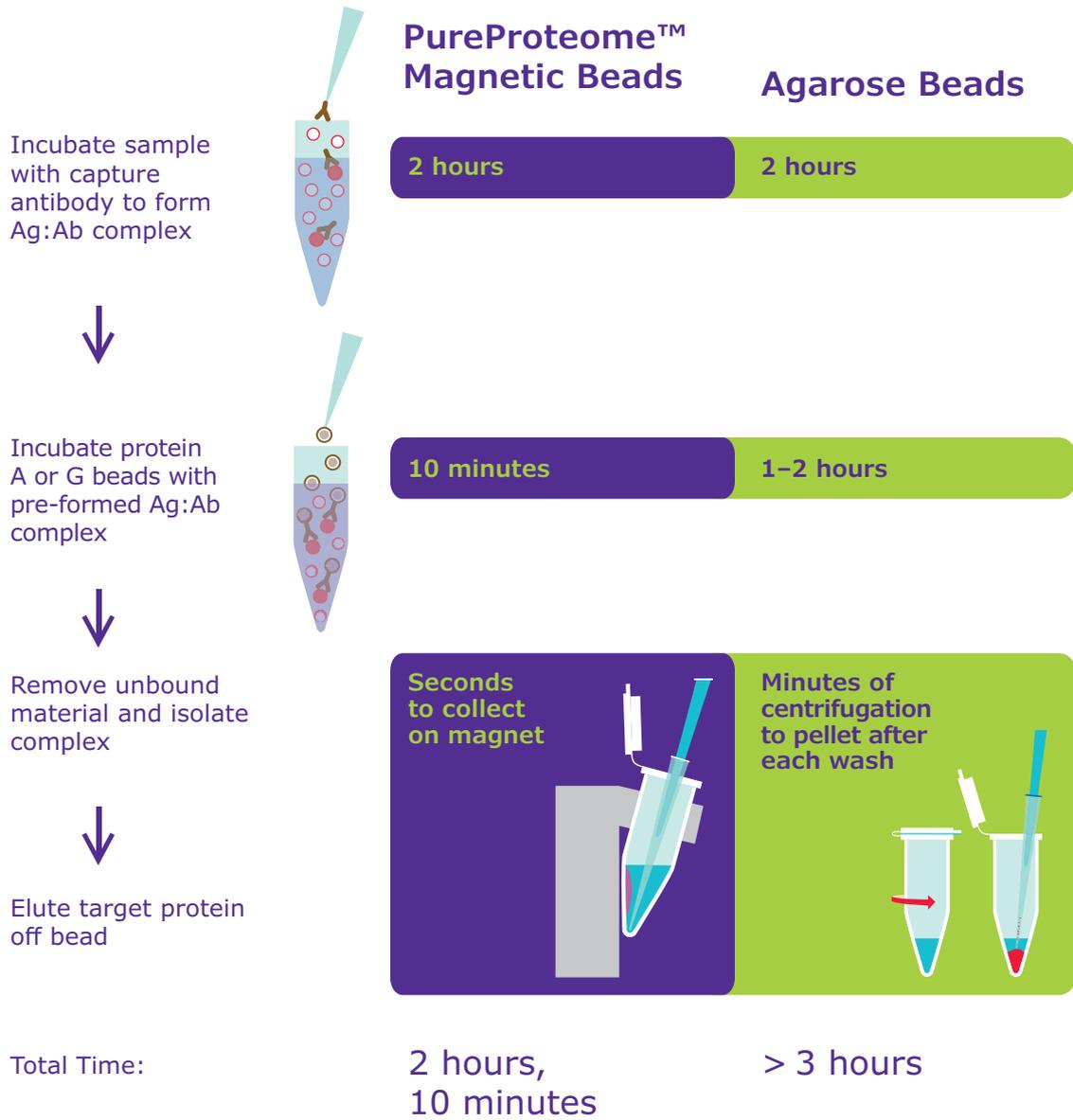
- **Flexible:** Choice of 0.3 µm, 1 µm or 2.5 µm COOH magnetic beads
- **Automation-Compatible:** Smaller beads have higher buoyancy properties while retaining strong magnetic capability

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

PureProteome™ Beads

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.

**Excellent yields.
Exceptional purity.
Faster protocol.**



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 Lambda Magnetic Beads bind to the lambda light chain constant region. 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 F(ab) and F(ab')₂.

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

Relative Affinity

	Protein A/G Mix	Protein A	Protein G	Kappa Ig Binder	Lambda Ig Binder	Kappa/Lambda Mix*
Antibodies						
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	●	●	●			

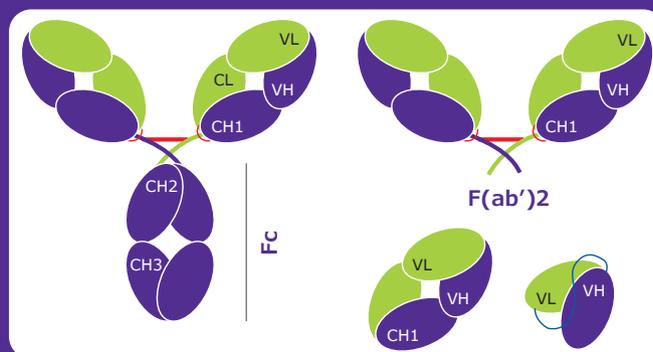
* PureProteome™ Kappa/Lambda mix is not a catalog item. Simply procure the Kappa and Lambda beads individually and mix at a 1:1 ratio.

	A/G Mix	Protein A	Protein G	Kappa Ig Binder	Lambda Ig Binder	Kappa/Lambda Mix*
Fragments						
Human I					●	●
Human k				●		●
Human Fc	●	●	●			
Human scFv	●	●		●	●	●
Human F(ab') ₂	●	●	●	●	●	●
Human F(ab)	●	●	●	●	●	●

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, and will not bind the scFv.



Ordering Information

Application	Description	Fisher Scientific Cat. No.
IP, Antibody Purification, F(ab) 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**	LSKMAG1C10
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. Agarose that combines Protein A and Protein G provides the binding characteristics of both in a single reagent.

Ordering Information

Description	Size	Fisher Scientific Cat. No.
Protein A Agarose	1.5 mL	IP0215ML
	10 mL	16-125
Protein G Agarose	1.5 mL	IP0415ML
	10 mL	16-266
Protein A + Protein G Agarose	1.5 mL	IP05-1.5ML
	10 mL	IP1010ML

Affinity Purification with Recombinant Fusion Tags

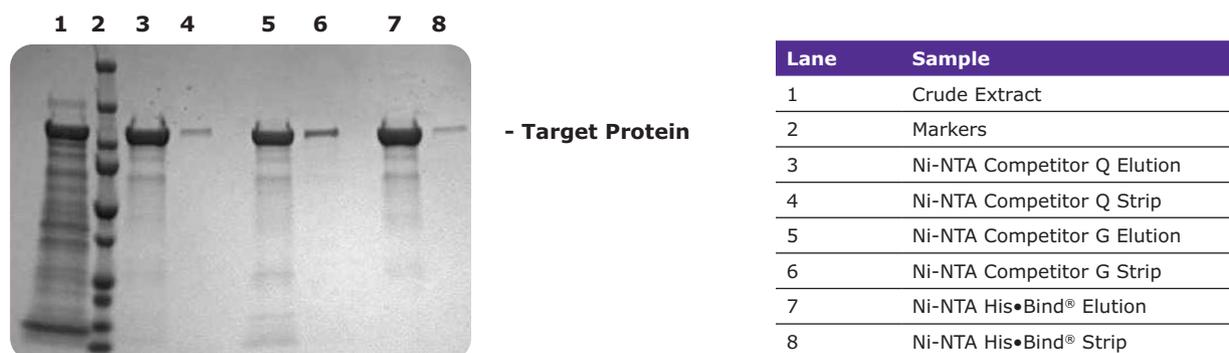
Recombinant protein purification by tag-specific affinity chromatography is a proven technology that results in highly specific recognition and purification of recombinant proteins.

His•Tag® Purification

Ni-NTA His•Bind® Resin has a binding capacity of over 10 mg of His-Tagged fusion protein per mL resin.

The agarose matrix on the Ni-NTA His•Bind® Superflow™ Resin is structured with more crosslinking for enhanced bead rigidity, for exceptional compatibility 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.



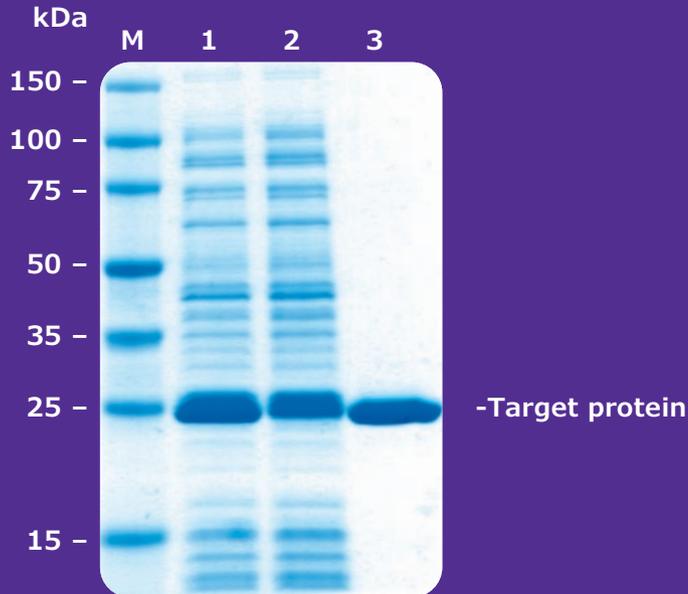
Ni-NTA His•Bind® performance vs. equivalent competitor resins Vector pET-28b (+) was used to express a His-Tag fusion protein of 119KDa in E. coli BL21 (DE3) cells, induced culture was processed with BugBuster® Master Mix, and protein extract was divided evenly to proceed to the His-Tag purification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

Ordering Information

Application	Description	Fisher Scientific Cat. No.
Ni-NTA His•Bind® Resin		
Small to medium scale	Ni-NTA His•Bind® Resin	70-666-3
Gravity flow column	BugBuster® Ni-NTA His•Bind® Purification Kit	70-751-3
Recommended for eukaryotic extracts	Ni-NTA Buffer Kit	70-899-3
Ni-NTA His•Bind® Superflow™ Resin		
Small to production scale	Ni-NTA His•Bind® Superflow™ Resin	70-691-3
FPLC or gravity flow column	Ni-NTA Buffer Kit	70-899-3
Uncharged IDA His•Bind® Resin		
Uncharged (metal flexibility)	IDA His•Bind® Resin	69-670-3
Reusability	His•Bind® Buffer Kit	69-755-3
Small to medium scale	His•Bind® Purification Kit	70-239-3
Gravity flow column or batch mode	BugBuster® His•Bind® Purification Kit	70-793-3

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 exceptional binding capacity.



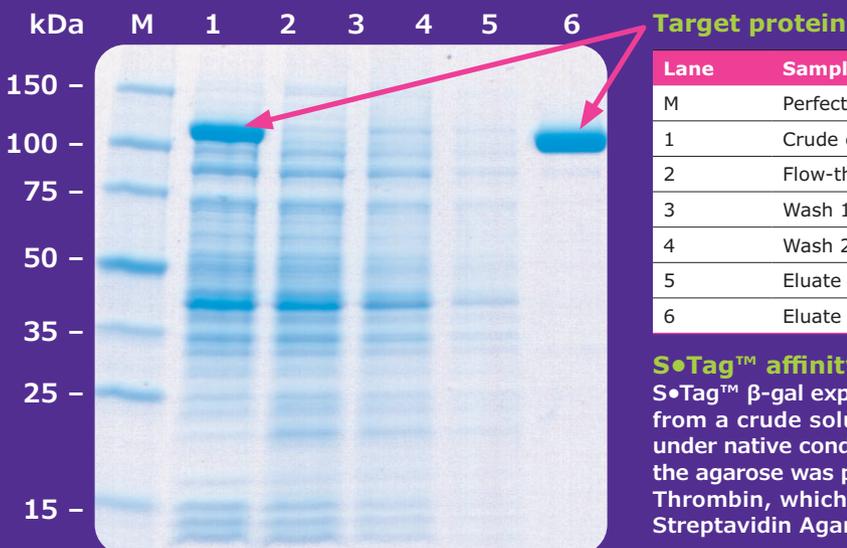
Lane	Sample
M	PerfectProtein™ markers 15–150 kDa
1	BugBuster® extract
2	Flow-through
3	Eluate

GST•TAG™ Purification

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

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.



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® epitope — 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

Description	Fisher Scientific Cat. No.	
GST•Tag™ Purification		
GST•Bind™ Resin	70-541-5	
BugBuster® GST•Bind™ Purification Kit	70-794-3	
S-Tag Purification		
S-protein Agarose	69-704-3	
S•Tag™ Thrombin Purification Kit	69-232-3	
S•Tag™ rEK Purification Kit	69-065-3	
Strep•Tag® II Purification		
Strep-Tactin® Superflow Agarose	71-592-3	
Strep-Tactin® Buffer Kit	71-613-3	
Strep-Tactin® SpinPrep Kit	71-608-3	
D-Desthiobiotin	71-610-3	
T7•Tag® Purification		
T7•Tag® Affinity Purification Kit	69-025-3	
T7•Tag® Antibody Agarose	69-026-3	
Streptavidin Agarose		
Description	Size	Fisher Scientific Cat. No.
Streptavidin Agarose	10 mL	16-126

Protein Depletion

ProteoExtract® Agarose Columns

Human serum and plasma samples are rich sources of proteomic information, reflecting processes regulating normal or diseased states. Today's ultra-sensitive analytical methods, such as two-dimensional (2D) gel electrophoresis and mass spectrometry, can detect minute changes in expression profiles — but ultrasensitive approaches typically require the removal of highly abundant proteins (HAP) and moderately abundant proteins (MAP).

We offer a range of kits and resins for depleting high-abundance proteins (HAP) from serum or plasma samples. Choose from the PureProteome™ magnetic bead kits and resins or the ProteoExtract® agarose columns. First, identify the species of your serum/plasma source — the following tables summarize the different solutions for your needs.

Ordering Information

Description	Format	Species	Proteins Depleted	Fisher Scientific Cat. No.
ProteoExtract® Albumin/IgG Depletion Kit	Agarose columns	Human, rabbit, rat, mouse, pig and bovine	Albumin and IgG > 80 %	12-264-21KIT
ProteoExtract® Albumin Depletion Kit	Agarose columns	Human, rabbit, rat, mouse, pig and bovine	Albumin > 80 %	12-264-01KIT

Application	Description	Fisher Scientific Cat. No.
Depletion/Enrichment	PureProteome™ Albumin Magnetic Beads	LSKMAGL10
	PureProteome™ Albumin/IgG Depletion Kit	LSKMAGD12
	PureProteome™ Human Albumin/Immunoglobulin Depletion Kit*	LSKMAGHDKIT

*Human only.

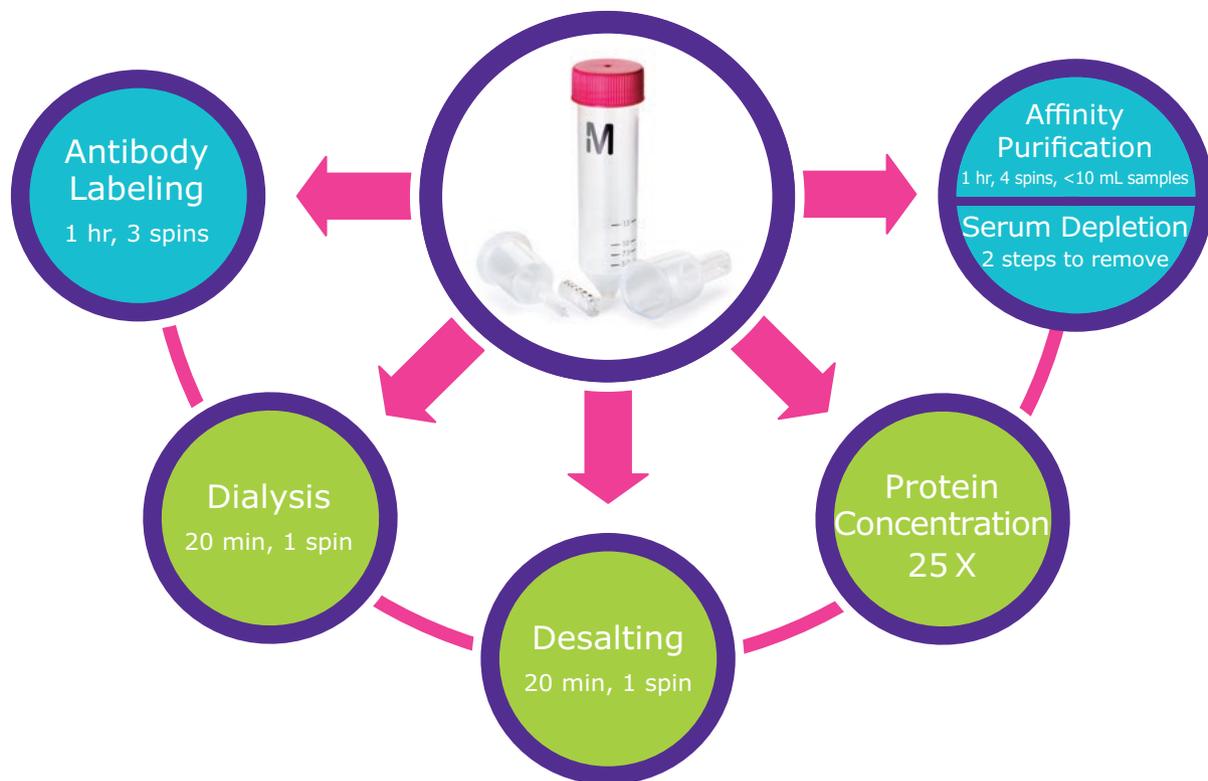
Amicon® Pro Purification System

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 using 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 while reducing hands-on time.

Whether you need to affinity purify, concentrate, dialyze, or any combination of the three, the Amicon® Pro Purification System will save 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

Exchange Device
Just one spin required with the large 10 mL chamber for the pre-wash, bind, wash, elute and buffer exchange steps

Exchange Tip
Continuous buffer exchange achieved with the unique exchange tip design



Frit
All-in-one device achieved with the frit holding back the affinity purification resin

Amicon® Ultra 0.5 mL Filter
Gentle elution and concentration of your protein sample in a single spin enabled by the Amicon® Ultra 0.5 mL filter

Ordering Information

To choose the appropriate Amicon® Pro device, determine the nominal molecular weight cut-off (NMWCO) for 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.

"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

Description

Amicon® Pro Purification Kits 12/pk Includes reagent kit (resin and buffers)	Reagent Kit Only	NMWCO				
		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	NMWCO				
	3,000	10,000	30,000	50,000	100,000
Amicon® Pro Purification System Trial Pack 2/pk	ACS500302	ACS501002	ACS503002	ACS505002	ACS510002
Amicon® Pro Purification System 12/pk	ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Purification System 24/pk	ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

Amicon® Pro Purification System 24/pk without Amicon® Ultra 0.5 mL filter:

ACS500024

Protein Purification with Protease Cleavage Enzymes

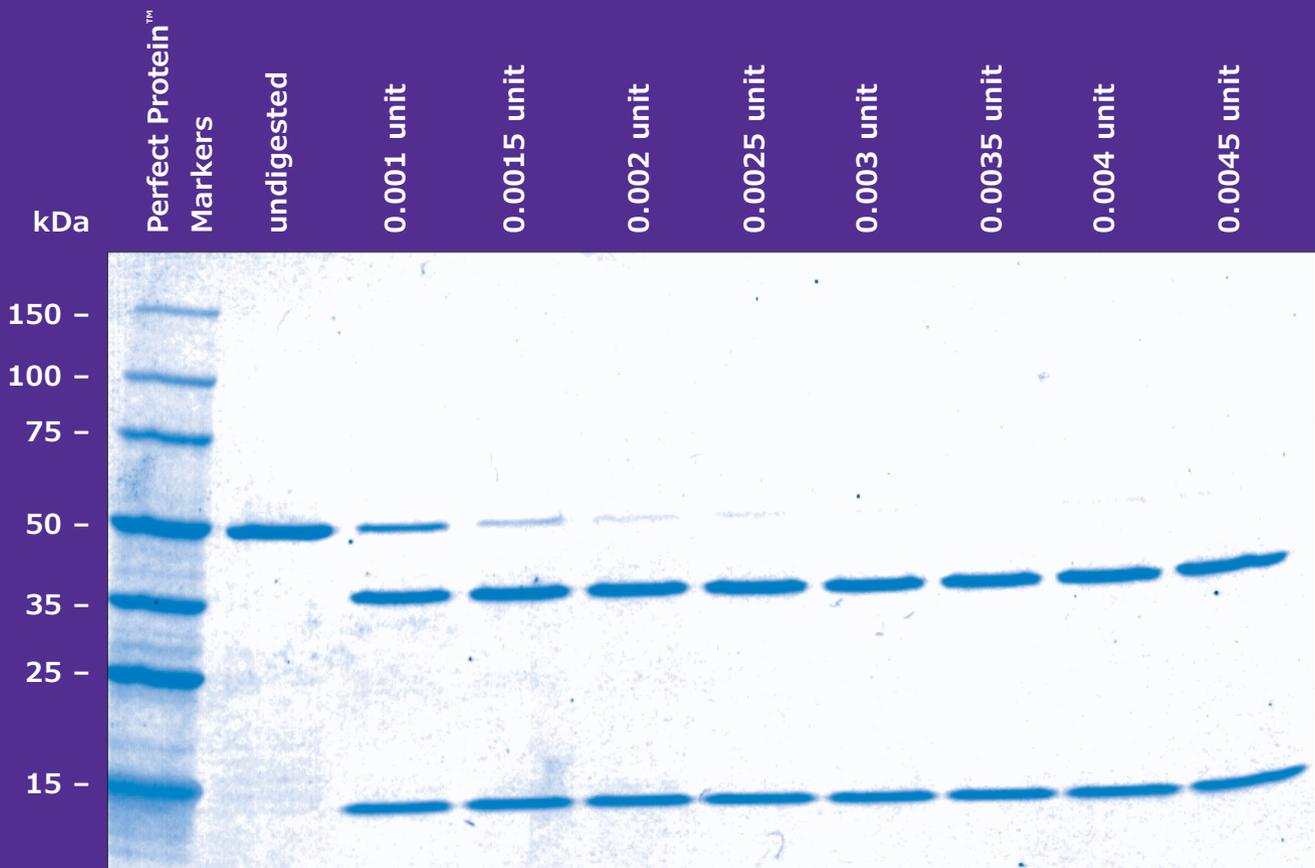
Featured Products

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 includes not only 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 μ g of Cleavage Control Protein in an overnight digestion. Samples were analyzed by SDS-PAGE (4–20% gradient gel) followed by staining with Coomassie® blue stain. The 0.0045 unit lane represents a 2.25-fold over-digestion.

HRV 3C Protease

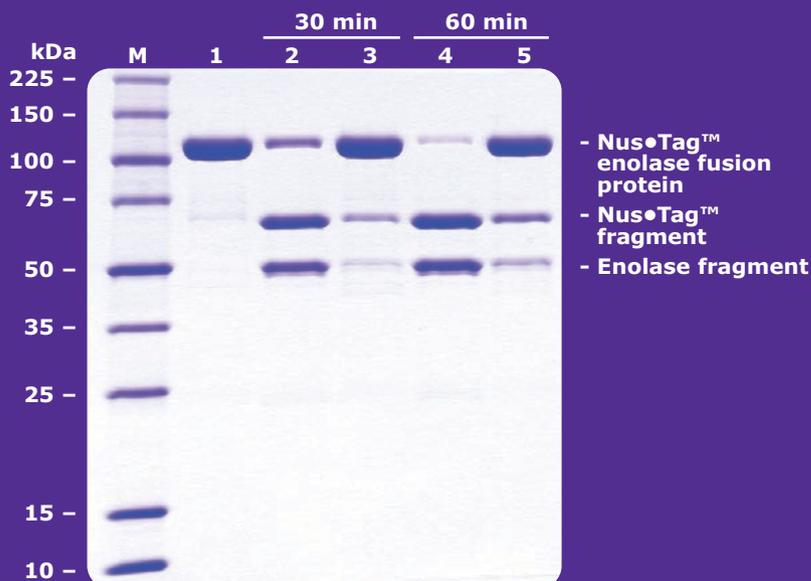
Highly efficient, specific cleavage of fusion proteins

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 were 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

Description	Cat. No.
Restriction-Grade Thrombin	69-671-3
Biotinylated Thrombin	69-672-3
Thrombin Cleavage Capture Kit	69-022-3
Restriction Grade Factor Xa	69-036-3
Factor Xa Cleavage Capture Kit	69-037-3
Recombinant Enterokinase	69-066-3
Enterokinase Cleavage Capture Kit	69-067-3
HRV 3C Protease	71-493-3

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 our 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.

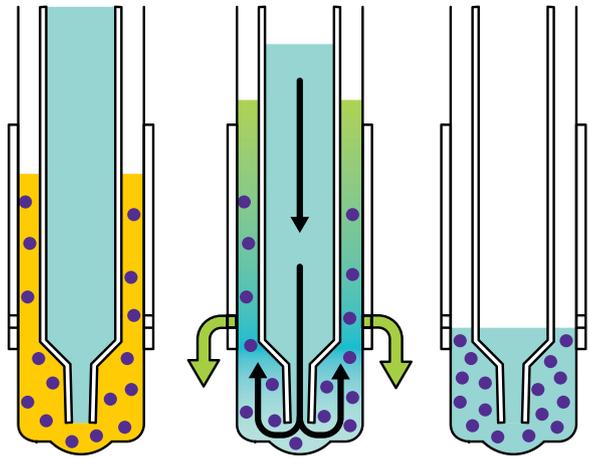
Fast: single spin

Gentle: unique design provides continuous diafiltration

Less Buffer: only 1.5 mL buffer required (given 0.5 mL initial sample)



Only 1 Spin



Add 1.5ml Buffer

Continuous flow
buffer exchange

Final 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.

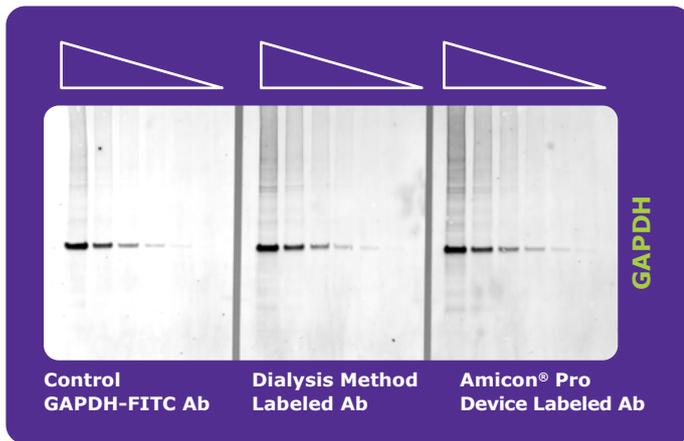
The gentleness of dialysis with the efficiency of diafiltration.

	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 with the Amicon® Pro system.

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 nominal molecular weight cut-off (NMWCO) for your protein of interest and your desired affinity purification scheme.

Amicon® Pro purification system – No Reagents Included	NMWCO				
	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
Amicon® Pro Purification System 24/pk without Amicon® Ultra 0.5 mL filter	ACS500024				

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

Product			D-Tube™ Dialyzer Mini	D-Tube™ Dialyzer Midi	D-Tube™ Dialyzer Maxi	D-Tube™ Dialyzer Mega	D-Tube™ Dialyzer Mega
Proteins/DNA/RNA/ Oligonucleotides	Molecular Weight Cut-off	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	MWCO (Da)	Qty/pk					
MW < 7 k	3,500	10		71-506-3	71-508-3	71-739-3	71-742-3
		50				71-739-4	71-742-4
7 < MW < 24 k	7,000	10	71-504-3	71-507-3	71-509-3	71-740-3	71-743-3
		50				71-740-4	71-743-4
24 k < MW	13,000	1 plate of 96	71-712-3				
		10	71-505-3		71-510-3		
		50					
		1 plate of 96	71-713-3				
	Floating Rack	Product (Qty/pk)	Mini (10)	Midi (10)	Maxi (10)	Mega (10)	Mega (10)
			71-512-3	71-513-3	71-514-3	71-748-3	71-748-3

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 Centrifugal Filters 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



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

Ultracel® Low-binding Membranes

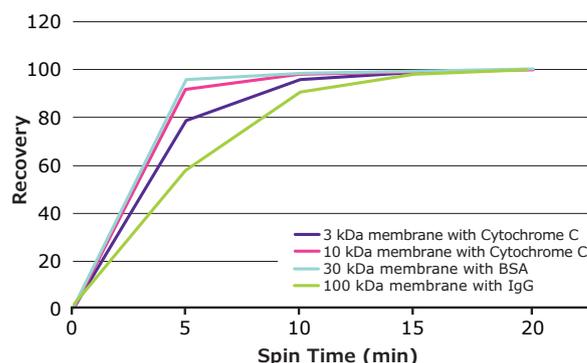
- Vertical membrane design aligns with filtrate rather than perpendicular for less clogging, less waste, and faster filtration
- Ultra-fast sample processing achieving concentration in as little as 10 minutes
- 25- to 80-fold concentration in a single step

Broad Chemical Compatibility

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

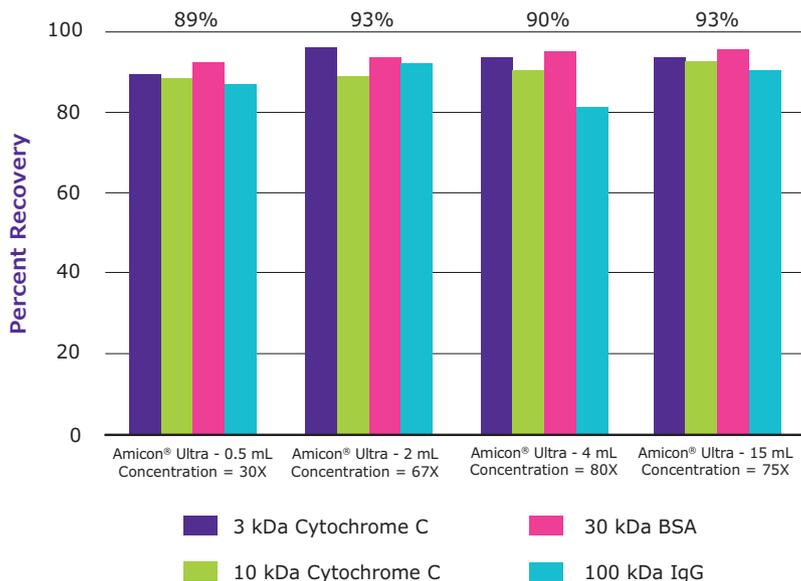
Reliable Samples

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



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

Amicon® Ultra 4 mL filters were tested for percent recovery and spin time.



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 devices) were tested (3 kDa membrane with Cytochrome C, 10 kDa membrane with Cytochrome C, 30 kDa membrane with BSA and 100 kDa membrane with 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 nominal molecular weight cutoff (NMWCO).

	Starting Volume	< 0.5 mL	< 2 mL	< 4 mL	< 15 mL
Molecular Weight (MW)	Proteins	NMWCO (Da)			
	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
Length	Single-Stranded and Double-Stranded Nucleic Acids	NMWCO (Da)			
	137–1159 bp	30,000	30,000	30,000	30,000
Particle Diameter (DIA)	Nanoparticles	NMWCO (Da)			
	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

NMWCO: Nominal Molecular Weight Cut Off

10,000 NMWCO Amicon® Ultra-4 and -15 filters are both CE marked and registered 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.

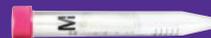
**Amicon®
Ultra-0.5
filter**



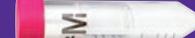
**Amicon®
Ultra-2
filter**



**Amicon®
Ultra-4
filter**



**Amicon®
Ultra-15
filter**



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

NMWCO (Da)				
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
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Amicon® Ultra Centrifugal Filters

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



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, allow the device to accommodate several wash steps with minimal sample loss.



Microcon® DNA Fast Flow Filter 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.

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		•	•

Microcon® Filter 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

Ordering Information

Description	Volume, mL	Min. final concentrate volume, µL	Qty/Pk	Fisher Scientific Cat. No.
Microcon® filter, Ultracel®-10 membrane, 10 kDa	0.5	5-50	100	MRCPRT010
Microcon® filter, Ultracel®-30 membrane, 30 kDa	0.5	5-50	100	MRCF0R030
Microcon® DNA Fast Flow Centrifugal Filter with Ultracel® membrane	0.5	5-50	100	MRCF0R100
Microcon® DNA Fast Flow PCR Grade filter with Ultracel® membrane, dual cycle EtO treated	0.5	5-50	20	MRCF0R100ET

Ultrafree® 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:

- 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 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)

Ordering Information

	Pore Size (µm)	Color	Sterility	Qty/Pk	Fisher Scientific Cat. 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)
	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)
	6	Dark Green	Non-sterile	100	UFC30SV00
Ultrafree®-CL Filter	0.1	Orange	Non-sterile	25	UFC40VV25
				100	UFC40VV00
	0.22	Yellow	Non-sterile	25	UFC40GV25
				100	UFC40GV00
				Sterile	50 (5 x 10)
	0.45	Red	Non-sterile	25	UFC40HV25
				100	UFC40HV00
	0.65	Purple	UFC4DV25	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	UFC30LH25MI
Ultrafree®-CL Filter	0.22	Yellow	Non-sterile	25	UFC40LG25
	0.45	Red	Non-sterile	25	UFC40LH25

Clinical Ultrafiltration

Separate free from protein-bound solute with Centrifree® filters

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, etc.
- Binding studies
- New drug investigations
- Deproteinization



Ordering Information

Description	Volume, mL	Min. final concentrate volume, μ L	MWCO (kDa)	Qty/Pk	Fisher Scientific Cat. No.
Centrifree® Ultrafiltration device with Ultracel® PL membrane	1	50	30	50	4104MI

Centrifree® filters are registered for *in vitro* diagnostic use.

Large Volume Concentration

Concentration of proteins and viruses

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, or precipitation techniques.

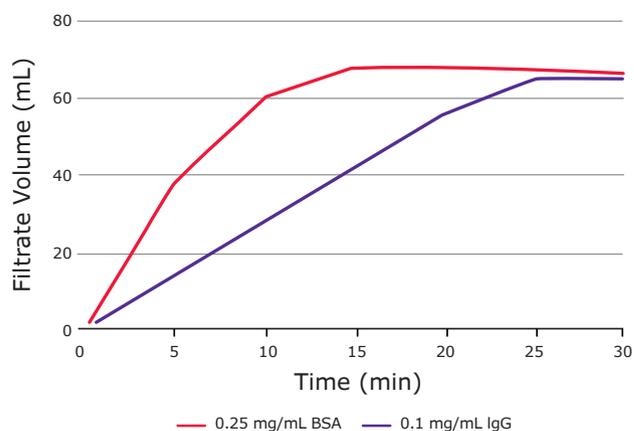
Centricon® Plus-70 filter advantages and applications

- >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 (Ultracel® PL-30 membrane at 3500 xg)



Ordering Information

Description	Volume, mL	Min. final concentrate volume, µL	NMWCO	Qty/Pk	Fisher Scientific Cat. No.
Centricon® Plus-70 3K filter	70	350	3	8	09-720-508
Centricon® Plus-70 10K filter	70	350	10	8	UFC701008
Centricon® Plus-70 30K filter	70	350	30	8	UFC703008
Centricon® Plus-70 100K filter	70	330	100	8	UFC710008

Amicon® Stirred Cells

50 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 nominal molecular weight cut-off (NMWCO) 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.
- Three different sizes to handle volumes from 50 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 three sizes

Max. Working Volume	Fisher Scientific Cat. No.
50 mL	UFSC05001
200 mL	UFSC20001
400 mL	UFSC40001

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.

Membrane Discs for Use in Stirred Cells

Ultracel® cut disc membranes

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.

Biomax® cut disc membranes

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.

Durapore® cut disc membranes

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.

Stirred Cell Accessories Expand Your Capabilities.

Amicon® Stirred Cell Selector Valve (Fisher Scientific Catalog No. 6003)

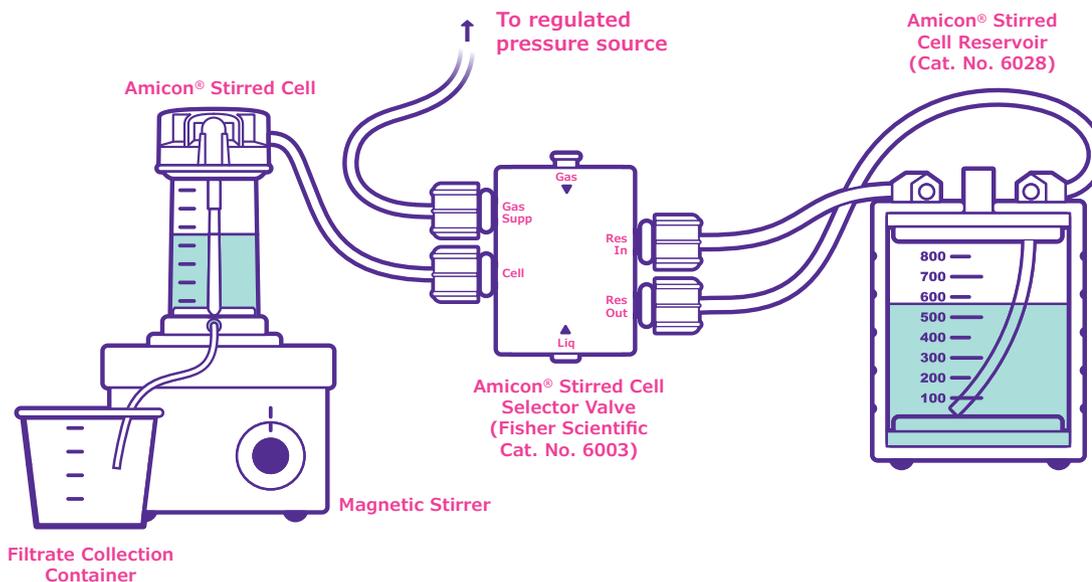
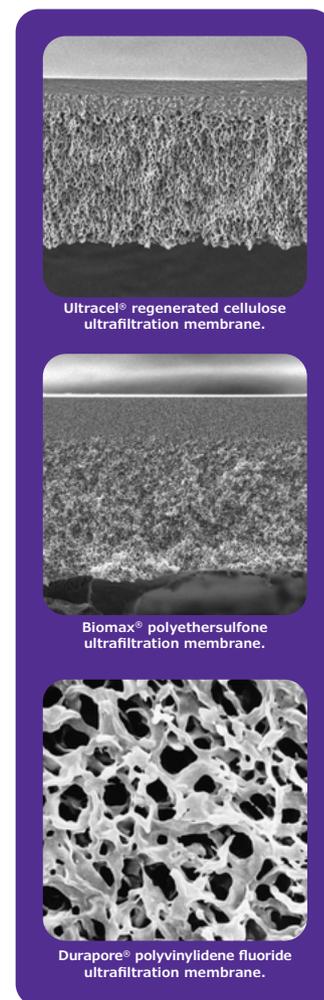
Valve with sliding control for instant switching from concentration to diafiltration, or switching gas and liquid lines simultaneously. Simplifies operation and avoids the need for multiple T-fittings and valves.

Amicon® Stirred Cell Manifold (Fisher Scientific Catalog No. 6015)

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 (Fisher Scientific Catalog No. 6028)

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.



Amicon® Stirred Cell setup for large volume concentration and continuous diafiltration using selector valve and reservoir accessories.



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