

MILLIPORE

protein purification, concentration  
and analysis for protein chemists





When a few microliters of protein represent days of work, years of research, and, ultimately, millions of dollars, every drop counts.

## Count on Millipore

### A long-standing commitment to protein research

Since the development of Immobilon®-P Transfer Membrane nearly two decades ago, Millipore scientists have remained committed to the design of quality products for protein scientists. The result is a broad product line and constant developments to support your research.

### High performance, innovative products

From our family of Amicon® ultrafiltration devices, to ZipTip® Pipette Tips for microvolume purification, to our superior line of Immobilon Transfer Membranes, Millipore provides trusted products for use in the concentration, purification and characterization of your samples.

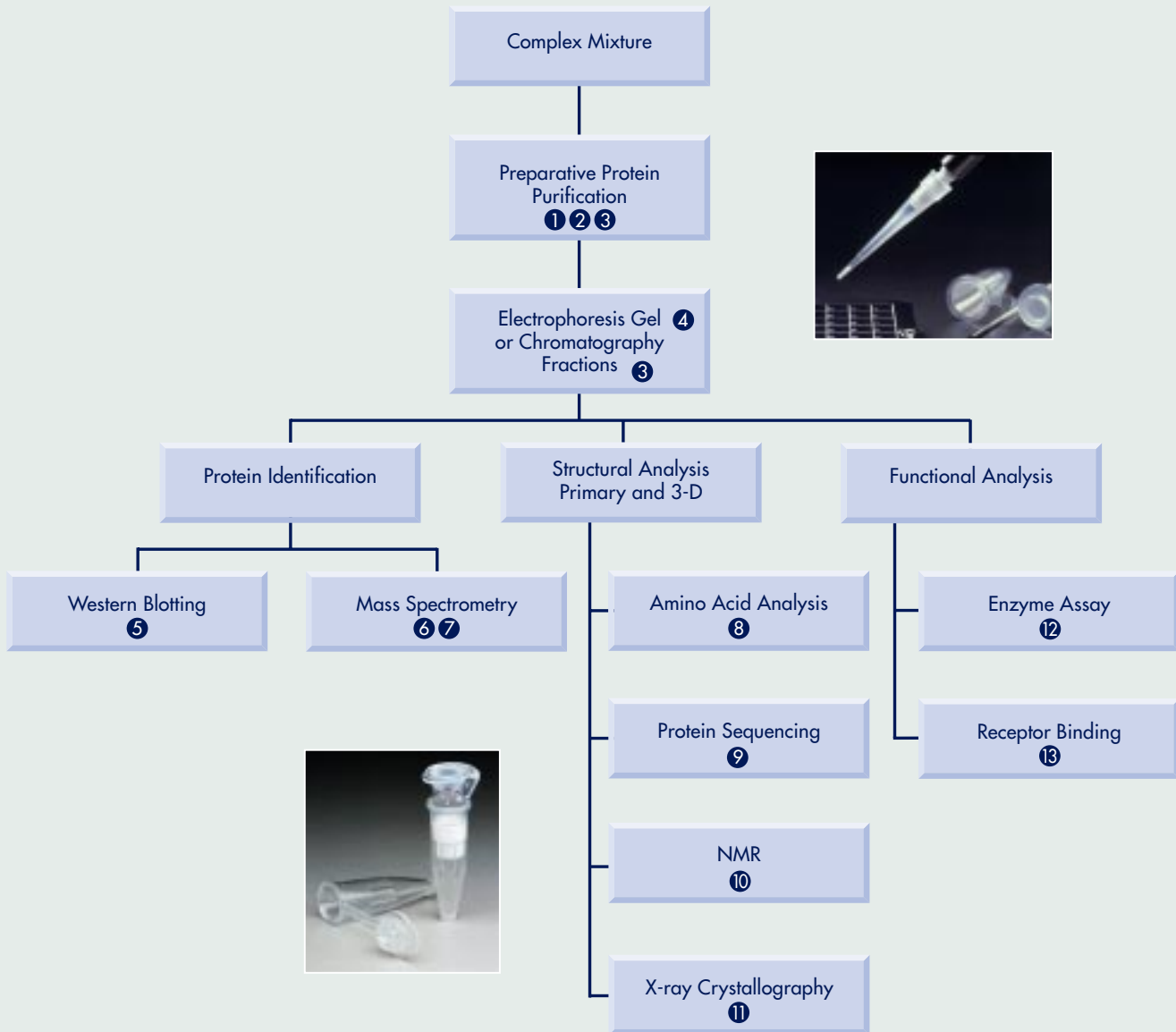
### Amicon family of devices

With the acquisition of Amicon in 1997, Millipore has been able to offer the most complete ultrafiltration product line to support a broad range of application needs for the concentration and purification of proteins.



### Unmatched customer support

Each Millipore product is backed by experienced technical applications specialists and an expansive library of protocols, application notes, user guides, specifications and frequently asked questions. Resources are available online or through the Millipore office nearest to you (see back cover for phone numbers).



## Guide to Applications

- ① **Millex™ Filter Units:** Particulate removal prior to HPLC
- ② **Microcon® YM-10:** Protein removal prior to C<sub>18</sub> HPLC  
**Microcon-SCX:** Remove detergents and salt prior to C<sub>18</sub> HPLC
- ③ **Amicon Ultrafiltration Devices:** Concentration and desalting of column fractions
- ④ **Centrilotor®:** Electroelution of protein from electrophoresis gels
- ⑤ **Immobilon-P and Immobilon-PSQ:** Blotting and immunodetection
- ⑥ **ZipTip Pipette Tips:** Concentration and sample prep before Mass Spectrometry
- ⑦ **Immobilon-P and Immobilon-PSQ:** Blotting and direct MS analysis
- ⑧ **Microcon-SCX:** Remove amine containing buffers prior to amino acid analysis  
**Microcon YM-3:** Remove partially hydrolyzed proteins
- ⑨ **Immobilon-PSQ and Immobilon-CD:** Electrotransfer or spotting for protein sequencing
- ⑩ **Amicon Ultrafiltration Devices:** Concentrate and exchange H<sub>3</sub>O for H<sub>2</sub>O
- ⑪ **Amicon Ultrafiltration Devices:** Concentrate and exchange buffers prior to crystal formation
- ⑫ **MultiScreen™:** Enzyme or cell based assays
- ⑬ **MultiScreen:** Protein and receptor binding assays

# There's nothing to lose with Millipore on your side

Millipore offers a complete range of ultrafiltration membranes and devices for sample concentration, purification, crude fractionation, and desalting or buffer exchange of soluble macromolecules.

## High recovery Ultracel™ membranes in every device

The Amicon device family is made exclusively with Ultracel regenerated cellulose membranes for maximum sample recovery. The Ultracel ultrafiltration membranes are recommended for a broad range of sample types and are ideal for concentrating and desalting extremely dilute solutions. The tight hydrophilic microstructure of Ultracel membranes ensures the lowest possible adsorption of protein and other macromolecules, and the highest recovery of fully functional proteins.

The protein retention (or membrane rejection) characteristic of the membrane is typically rated at greater than 95% of a globular solute with that molecular weight limit.

## Maximum recovery characteristics

The unique Amicon device design, with a built-in dead-stop and patented invert spin, allows for maximum recovery of your final concentrated sample.



## Technical literature

- Also available online at [www.millipore.com/amicon](http://www.millipore.com/amicon)
- Amicon Family Centrifugal Devices Brochure (FF021)
  - Ultrafiltration Selection Guide (PS009)
  - Desalting and Buffer Exchange with Millipore Centrifugal Devices Application Note (AN001EN00)
  - Microcon Centrifugal Filter Devices Data Sheet (PF185)
  - Centrificon Data Sheet (PF461EN00)
  - Centrificon Plus Centrifugal Filter Devices Data Sheet (PF188)
  - Amicon Centriplus Centrifugal Filter Devices Data Sheet (PF462)

# Amicon Concentrator Centrifugal Devices

## Selection Guide

### Ultracel Membranes

Molecular weight cutoffs range from 3,000 to 100,000 and are optimized for a range of applications to provide the highest possible sample recoveries.

Membrane NMWL	Protein Solute MWCO	UF Rejection* (%)	Retentate Recovery** (%)	Applications
3,000	Protamine Sulfate 5 – 10,000	95 – 100	85 – 90	<ul style="list-style-type: none"> <li>• Protein concentration</li> <li>• Protein purification</li> <li>• Desalting of column fractions</li> <li>• Peptide concentration and desalting/buffer exchange</li> <li>• Removal of partially hydrolyzed proteins</li> </ul>
	Cytochrome c 12,400	100	95 – 100	
5,000	Protamine Sulfate 5 – 10,000	78 – 80	55 – 60	<ul style="list-style-type: none"> <li>• Protein concentration</li> <li>• Protein purification</li> <li>• Desalting of column fractions</li> </ul>
	Cytochrome c 12,400	100	95 – 100	
10,000	Cytochrome c 12,400	100	95 – 100	<ul style="list-style-type: none"> <li>• Protein concentration</li> <li>• Protein purification</li> <li>• Desalting of column fractions</li> <li>• Protein isolation from cell lysates</li> <li>• Bound vs. free drugs from serum/plasma (protein removal)</li> <li>• Protein removal prior to C<sub>18</sub> HPLC</li> </ul>
30,000	$\alpha$ -chymotrypsinogen 25,000	97 – 100	80 – 85	<ul style="list-style-type: none"> <li>• Protein concentration</li> <li>• Protein purification</li> <li>• Desalting of column fractions</li> <li>• Protein isolation from cell lysates</li> <li>• Antibody concentration</li> <li>• Bound vs. free drugs from serum/plasma (protein removal)</li> </ul>
	BSA 67,000			
50,000	BSA 67,000	98 – 100	95 – 100	<ul style="list-style-type: none"> <li>• Protein concentration</li> <li>• Protein purification</li> <li>• Desalting of column fractions</li> <li>• Antibody concentration</li> </ul>
	Phosphorylase b 97,400	98 – 100	90 – 95	
100,000	Phosphorylase b 97,400	90 – 95	80 – 85	<ul style="list-style-type: none"> <li>• Protein concentration</li> <li>• Protein purification</li> <li>• Desalting of column fractions</li> <li>• Antibody concentration</li> </ul>
	IgG Fr II 156,000	97 – 100	90 – 95	

\* UF rejection is the percentage of product/solute that is retained by the UF membrane  
 \*\* % retentate recovery is the mass of the total product recovered in the retentate (concentrated product yield), or the percentage of product actually recovered in the concentrate

### Amicon Centrifugal Devices

Maximize sample recovery with Microcon, Centricon®, Centriplus® and the high throughput Centricon-Plus devices.

The Amicon devices are available in volume segments for samples less than 0.5 mL up to 80 mL volumes.

	Microcon <sup>†</sup>	Centricon	Centriplus	Centricon Plus-20	Centricon Plus-80
Maximum starting volume (mL)	0.5	2	10 – 15	20	80
Minimum concentrate volume (µL)	5 – 15	25	500	200	300
Centrifuge rotor type and size (mL)	Fixed angle 1.5	Fixed angle 15	Swinging bucket or fixed angle 50	Swinging bucket 50	Swinging bucket 250
Maximum centrifugal force (x g)	14,000	5,000	3,000	4,000	3,500
Active Filtration area (cm <sup>2</sup> )	0.34	0.92	2.34	10.33	19

<sup>†</sup>Also available in 96-place holder Microcon-96

# Cleaner Spectra for Better Identification and Characterization

Use ZipTip Pipette Tips to easily purify femtomoles to picomoles of proteins in seconds before mass spectrometry. Available with a variety of resins to meet your application needs, ZipTip Pipette Tips are a fast and simple method for microvolume purification prior to analysis.

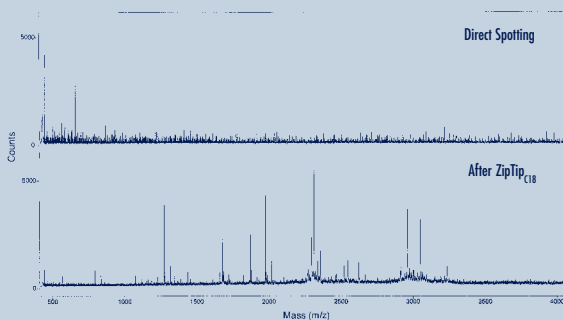
## One device for sample preparation and liquid handling

ZipTip is a 10  $\mu\text{L}$  pipette tip with a microvolume bed of reversed-phase, or affinity media fixed at its end with no dead volume. Use ZipTip Pipette Tips for concentrating, desalting, fractionating, and enriching 1.0  $\mu\text{L}$  – 100  $\mu\text{L}$  of protein or peptide sample prior to analysis.

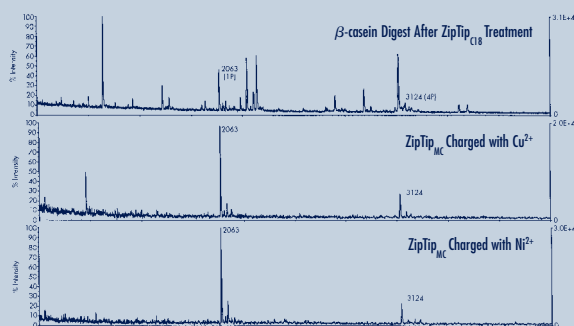
## Fast and simple

To bind sample, aspirate and dispense it through the resin several times. Then, wash away contaminants and unwanted biomolecules. Elute the concentrated, purified sample in 0.5  $\mu\text{L}$  to 4  $\mu\text{L}$  of compatible solvent and directly transfer it to a MALDI-TOF MS target, nanospray needle, or vial.

### Improved MALDI spectra for better identification...



### ...and better phosphopeptide characterization.





Prepare one sample in 60 seconds – or as many as 400 samples in 60 minutes

ZipTip Pipette Tips are compatible with these automated systems

- PE BioSystems SymBiot™ Sample Workstation for Biospectrometry™
- Genomic Solutions ProMST™ Sample Preparation Station
- Bruker Daltonics MAP™ II and MAP II/8 MALDI AutoPrep System
- Micromass® 2700-MS and MassPREP™ Sample Preparation Station
- CyBio CyBi™-Well 96 Automated Pipettor

Choose the ZipTip Pipette Tip for your application

ZipTip Pipette Tips will purify your sample prior to mass spectrometry, microbore HPLC, capillary electrophoresis, or other analytical techniques. They are compatible with single- or multi-channel pipettors, standard blunt-end HPLC needles, or automated liquid handling stations.

## C<sub>18</sub>/μ-C<sub>18</sub>

- Desalt and concentrate peptides, low molecular weight proteins, or oligonucleotides
- Step-fractionate complex peptide or protein mixtures for increased MALDI-TOF MS sensitivity and resolution
- Elute volumes as low as 0.5 μL with ZipTip<sub>μ-C18</sub>

## C<sub>4</sub>

- Desalt and concentrate low to intermediate molecular weight proteins and proteins >100,000 MW

## Metal Chelate (MC)

- Enrich phosphopeptides
- Purify 6xHis-tagged proteins

## Technical literature

Also available online at [www.millipore.com/ziptip](http://www.millipore.com/ziptip)

- ZipTip Data Sheet (PF172EN00)
- Protocol-Fractionation of Complex Peptide or Protein Mixtures Prior to MALDI-TOF MS Using ZipTip<sub>C18</sub>, ZipTip<sub>μ-C18</sub>, and ZipTip<sub>C4</sub> Pipette Tips (TN226)
- Protocol-Sample Preparation of Peptides or Proteins Prior to MALDI-TOF MS Using ZipTip<sub>C18</sub>, ZipTip<sub>μ-C18</sub>, and ZipTip<sub>C4</sub> Pipette Tips (TN224)
- Protocol-Sample Preparation of Peptides or Proteins Prior to Nanoelectrospray MS Using ZipTip<sub>C18</sub>, ZipTip<sub>μ-C18</sub> and ZipTip<sub>C4</sub> Pipette Tips (TN072)

- Protocol-Purification of 6xHis-tagged Proteins Prior to MALDI-TOF MS using ZipTip Pipette Tips (TN229)
- Protocol-Enrichment of Phosphopeptides Before MALDI-TOF and Nanoelectrospray MS Using ZipTip<sub>MC</sub> Pipette Tips (TN228)
- Recent Scientific Conference Poster Presentations also available online

# For superior results, use Immobilon Transfer Membranes

Life scientists have long relied on Millipore Transfer Membranes for protein blotting techniques. This family of high-performance membranes is optimized to deliver the results you need for effective protein analysis.

## Superior protein binding and high signal to noise ratio

Immobilon-P and Immobilon-PS<sup>Q</sup> Transfer Membranes are composed of polyvinylidene fluoride (PVDF) matrix that has been specifically developed and optimized for a wide range of protein chemistry

## Exceptional handling and staining characteristics

Immobilon Transfer Membranes are compatible with the solvents and harsh conditions used in protein sequencing and amino acid analysis as well as the solutions utilized in electrotransfer and detection protocols. The PVDF substrate allows easy handling without cracking or curling. The membrane can be cut without fracturing and will not tear under normal handling conditions. Immobilon-P and Immobilon-PS<sup>Q</sup> are compatible with chromogenic, chemiluminescent and chemifluorescent detection techniques.

## Improved performance over nitrocellulose

Membrane	pmol Bound Initially	% Retained	pmol Retained
Immobilon-P	36.0	80.2	28.9
Nitrocellulose	40.6	54	21.9

*The Immobilon-P membrane retains a higher proportion of electroblotted protein in standard immunodetection buffers.*

Unlike nitrocellulose, Immobilon-P and Immobilon-PS<sup>Q</sup> membranes also tolerate the high concentrations of methanol used in transfer buffers and ionic dyes without shrinking or altering the electrophoretic bands.

applications including immunodetection, rapid immunodetection and protein sequencing. It has also been referenced as a substrate for MALDI-TOF MS. PVDF is superior to other protein blotting membranes because it binds and retains proteins efficiently while remaining compatible with a variety of blocking protocols. Signal to noise ratios on PVDF are higher than those on other membrane substrates.

## Ideal surface for rapid immunodetection

The hydrophobic properties of Immobilon-P make it the ideal matrix for the Rapid Immunodetection Procedure developed by Millipore. Rapid Immunodetection eliminates the need for blocking the membrane prior to immunodetection on a western blot. As a result, the number and length of wash steps are drastically reduced to enable faster results without compromising specificity or sensitivity.



*Comparison of Rapid versus Standard Immunodetection of transferrin in a double dilution series of human serum using BCIP/NBT as the substrate on Immobilon-P Transfer Membrane.*

# Immobilon Transfer Membranes

## Optimized membranes

**Immobilon-P<sup>SQ</sup>**: The high surface area of this PVDF membrane maximizes protein binding making it the ideal substrate for high efficiency capture of peptides and proteins in the <20 kDa range. When transferring proteins in the 10 – 20 kDa range, both Immobilon-P and Immobilon-P<sup>SQ</sup> should be investigated for optimal signal.

**Immobilon-CD**: Use this cationically derivatized, hydrophilic PVDF membrane for optimal protein fragmentation and internal sequencing. Immobilized proteins are enzymatically digested and recovered fragments can be separated by HPLC or electrophoresis.

## Choose the Immobilon Transfer Membrane best suited for your application

Application	Immobilon-P	Immobilon-P <sup>SQ</sup>	Immobilon-CD
Western blotting	●	▲	
Low MW western blotting		●	
Reprobing on western blotting	●		
Amino acid analysis	●	■	
Glycoprotein detection	●		
Internal protein sequencing			●
Peptide mapping	■		●
Protein sequencing	■	●	■
Dot/Slot	●		

Key: ● excellent; ■ good; ▲ testing recommended prior to use

### Technical literature

Also available online at  
[www.millipore.com/immobilon](http://www.millipore.com/immobilon)

- Immobilon Brochure (FF250)
- Rapid Immunodetection Protocol (RP562)

- Protein Blotting Applications Guide (TP001)
- Rapid Immunodetection Method on Immobilon-P Using Chemiluminescence (TN051)
- Transillumination Application Note (AN031)

[www.millipore.com/immobilon](http://www.millipore.com/immobilon)

# Amicon Centrifugal Devices



Microcon

Model & Max. Volume	Membrane Type	MW Cut-off	8/pk	24/pk	100/pk	500/pk
Microcon 500 µL	Ultracel-YM					
	Regenerated cellulose	3,000	42420	42403	42404	—
		10,000	42421	42406	42407	42408
		30,000	42422	42409	42410	42411
		50,000	42423	42415	42416	—
100,000	42424	42412	42413	42414		



Centricon

Microcon-SCX 500 µL	Sulfonated polystyrene divinyl benzene		42460	42461	42462	—
Centricon 2 mL	Ultracel-YM					
	Regenerated cellulose	3,000	4240	4202	4203	—
		10,000	4241	4205	4206	4207
		30,000	4242AM	4208	4209	—
		50,000	4243	4224	4225	—
100,000	4244	4211	4212	4213		



Centriplus

Centriplus 10 – 15 mL	Ultracel-YM					
	Regenerated cellulose	3,000	4410	4420	—	—
		10,000	4411	4421	—	—
		30,000	4412	4422	—	—
		50,000	4413	4423	—	—
100,000	4414	4424	—	—		



Centricon Plus-20

Model & Max. Volume	Membrane Type	MW Cut-off	2/pk	8/pk	24/pk
Centricon Plus-20 20 mL	Ultracel-PL Regenerated cellulose	5,000	UFC2 LCC 02	UFC2 LCC 08	UFC2 LCC 24
		10,000	UFC2 LGC 02	UFC2 LGC 08	UFC2 LGC 24
		30,000	UFC2 LTK 02	UFC2 LTK 08	UFC2 LTK 24



Centricon Plus-80

Centricon Plus-80 80 mL	Ultracel-PL Regenerated cellulose	5,000	UFC5 LCC 02	UFC5 LCC 08	—
		10,000	UFC5 LGC 02	UFC5 LGC 08	—
		30,000	UFC5 LTK 02	UFC5 LTK 08	—

## ZipTip Pipette Tips



ZipTip

Resin	8/pk	24/pk	Catalogue Number	
			96/pk (96-place tip rack)	960/pk (10 x 96-place tip rack)
C <sub>18</sub>	ZTC1 8S0 08	ZTC1 8S0 24	ZTC1 8S0 96	ZTC1 8S9 60
μ-C <sub>18</sub>	ZTC1 8M0 08	ZTC1 8M0 24	ZTC1 8M0 96	ZTC1 8M9 60
C <sub>4</sub>	ZTC0 4S0 08	ZTC0 4S0 24	ZTC0 4S0 96	ZTC0 4S9 60
MC	ZTOM CS0 08	ZTOM CS0 24	ZTOM CS0 96	ZTOM CS9 60

## Immobilon Transfer Membranes

Immobilon-P (PVDF), 0.45 μm pore size



Immobilon

Dimensions	Qty/Pk	Catalogue No.
7 x 8.4 cm sheet	50	IPVH 078 50
9 x 12 cm sheet	10	IPVH 091 20
10 x 10 cm sheet	10	IPVH 101 00
15 x 15 cm sheet	10	IPVH 151 50
20 x 20 cm sheet	10	IPVH 202 00
26 x 26 cm sheet	10	IPVH 304 00
26.5 cm x 3.75 m roll	1	IPVH 000 10

Immobilon-P<sup>SQ</sup> (PVDF), 0.2 μm pore size

Dimensions	Qty/Pk	Catalogue No.
10 x 10 cm sheet	10	ISEQ 101 00
15 x 15 cm sheet	10	ISEQ 151 50
20 x 20 cm sheet	10	ISEQ 202 00
26 x 26 cm sheet	10	ISEQ 262 60
26.5 cm x 3.75 m roll	1	ISEQ 000 10

Immobilon-CD, 0.1 μm cationically derivatized, hydrophilic PVDF

Dimensions	Qty/Pk	Catalogue No.
26 cm x 3.75 m roll	1	ICDM 000 10

## To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:

In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**

In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**

On the Internet  
<http://www.millipore.com>

E-mail: [tech\\_service@millipore.com](mailto:tech_service@millipore.com)

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## Additional Products

### Microcon-SCX Microconcentrator

Microcon-SCX employs a strong cation exchange membrane for rapid concentration and/or purification of peptides, amino acids or DNA oligomers. It is ideal for removal of salts, detergents and other low-MW contaminants from samples before structural analysis. [Recommended literature: P446]

### Microcon-96 Multiwell Assemblies

The proven performance of Microcon filter units is available in a high throughput format. Microcon-96 assemblies are pre-loaded with 96 Microcon-YM Ultracel filter units for rapid centrifugation of 100 – 300 µL samples. [Recommended literature: PF1018EN00]

### Centrilutor Micro-Electroeluter

Centrilutor allows rapid and high yield recovery of small amounts of protein (1 to 25 µg) after electrophoretic separation. It is ideal for purification of very small quantities of proteins for automated protein sequencing, peptide mapping, and amino acid analysis. Centrilutor employs the use of the Centricon concentrator filter devices for sample collection during electroelution, and for protein concentration. [Recommended literature: PF008EN00]

### MultiScreen Filter Plates

MultiScreen 96-well filter plates are available in a broad range of plate and filter materials for applications including enzyme assays and protein and receptor binding assays. [Recommended literature: MM021, FF005EN00]

### Sample Prep Millex Filter Units

Use Millex syringe-driven filter units for particulate removal prior to HPLC. For protein solutions, use Millex with low-protein binding PVDF or PTFE membranes in 0.45 or 0.20 µm pore sizes. [Recommended literature: FF050EN00]

# MILLIPORE

## For Technical Assistance

Contact Millipore: 1-800-MILLIPORE  
(1-800-645-5476)

E-mail: [tech\\_service@millipore.com](mailto:tech_service@millipore.com)



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## Additional Products

### Microcon-SCX Microconcentrator

Microcon-SCX employs a strong cation exchange membrane for rapid concentration and/or purification of peptides, amino acids or DNA oligomers. It is ideal for removal of salts, detergents and other low-MW contaminants from samples before structural analysis. [Recommended literature: P446]

### Microcon-96 Multiwell Assemblies

The proven performance of Microcon filter units is available in a high throughput format. Microcon-96 assemblies are pre-loaded with 96 Microcon-YM Ultracel filter units for rapid centrifugation of 100 – 300  $\mu$ L samples. [Recommended literature: PF1018EN00]

### Centrilutor Micro-Electroeluter

Centrilutor allows rapid and high yield recovery of small amounts of protein (1 to 25  $\mu$ g) after electrophoretic separation. It is ideal for purification of very small quantities of proteins for automated protein sequencing, peptide mapping, and amino acid analysis. Centrilutor employs the use of the Centricon concentrator filter devices for sample collection during electroelution, and for protein concentration. [Recommended literature: PF008EN00]

### MultiScreen Filter Plates

MultiScreen 96-well filter plates are available in a broad range of plate and filter materials for applications including enzyme assays and protein and receptor binding assays. [Recommended literature: MM021, FF005EN00]

### Sample Prep Millex Filter Units

Use Millex syringe-driven filter units for particulate removal prior to HPLC. For protein solutions, use Millex with low-protein binding PVDF or PTFE membranes in 0.45 or 0.20  $\mu$ m pore sizes. [Recommended literature: FF050EN00]