# **Sample Preparation**



# Reagents and Consumables

#### Protein Sample Preparation

- Protein Extraction
- Affinity Chromatography
- Gelfiltration
- Ultrafiltration
- Protein Purification Kits
- Phosphatase Inhibitor Mixes
- Protease Inhibitor Mixes
- Protein Quantification
- Enzymes
- SERVA ICPL<sup>™</sup>-Kit

Nucleic Acid Sample Preparation

- Buffers & Enzymes
- Reagents and Solutions

# **Sample Preparation**

A first important step in the successful isolation and purification of proteins is the efficient lysis of cells and tissues. **SERVA's Mammalian Protein Extraction Kits** provide a fast and easy method for the isolation of native proteins from cells and tissues.

**Gelfiltration**, **ultrafiltration** or the highly selective **affinity chromatography** are the main tools for purification of proteins from supernatants of cell and tissue extracts. These methods are complemented by a selection of highly specific **enzymes** as well as **"empty" columns**. **Protease** and **phosphatase inhibitors** as mixes or stand-alone reagents are protecting the valuable proteins against degradation or modification.

For isolation of intact and pure nucleic acids SERVA offers highly active **enzymes, ready-to-use buffer solutions** and **reagents**, all guaranteed DNase and RNase free.

SERVA offers a broad range of **detergents** and **dialysis tubings** in different formats. For more information, please refer to the complementary brochures **"Detergents"** and **"Dialysis Tubings"**.

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# Protein Sample Preparation Protein Extraction

One of the most efficient buffers for the lysis of mammalian cells and tissues is **RIPA buffer**. Save time and labor by using SERVA's ready-to-use **RIPA Buffer** for extraction of total protein suitable for many applications like Western Blotting, protein purification and protein assays.

#### **RIPA Buffer**

Product	Size	Cat. no.
DIDA Duffer	100 ml	39244.01
RIPA Buffer	500 ml	39244.02

SERVA's Mammalian Protein Extraction Kits provide a fast and easy method for the isolation of total protein

- Mild but efficient lysis with high protein yield
- No need for mechanical disruption or ultracentrifugation

Mammalian Protein Extraction Kits

or native cytoplasmic, membrane and nuclear protein fractions from cells or tissues.

- Reagents are compatible with standard protein quantification assays like BCA Protein Assay
- Extracted proteins are directly applicable in downstream applications like Western Blot, ELISA, EMSA

#### Total proteins in only Mammalian Total Protein Extraction Kit 100 samples 39241.01 55 min Membrane and cyto-Mammalian Membrane Protein Extraction Kit 50 samples 39242.01 plasmic proteins in only 75 min. Nuclear and cytoplas-Mammalian Nuclear and Cytoplasmic Protein Extraction Kit mic proteins in only 50 samples 39243.01 80 min. 2 3 4 2 3 4 ß-Tubulin ß-Tubulin HDAC1

Fig. 1 Membrane proteins were isolated from HEK293 cells using the SERVA Mammalian Membrane Protein Extraction Kit and the Membrane Protein Extraction Kit of a competitor, following the respective protocols. To determine the percentage of cross contamination by cytoplasmic proteins in the membrane protein fraction,  $\mathcal{B}$ -Tubulin was detected in the cytoplasmic (lane 1,2) and membrane extract (lane 3,4) by Western blot.

Fig. 2 Cytoplasmic and nuclear proteins were isolated from HEK293 cells using the SERVA Mammalian Membrane Protein Extraction Kit and the Membrane Protein Extraction Kit of a competitor, following the respective protocols. To analyze the efficiency of cellular fractionation, ß-Tubulin and HDAC1 were detected in the cytoplasmic (lane 1,2) and nuclear extract (lane 3,4) by Western blot.

At SERVA we are committed to assisting you at every stage in your protein sample preparation procedures – SERVA Serving Scientists!

# Affinity Chromatography

Affinity chromatography is a technique that separates tagged proteins and other biomolecules using biological interac-

A. GST-Tag Purification

Fusion proteins expressed from pGEX vectors contain a Glutathione S-Transferase (GST) moiety and can therefore be purified tions. This technique is widely used to obtain high purity yield accompanied by good resolution and selectivity.

to near homogeneity by affinity chromatography with glutathione as a substrate.

**The Glutathione Agarose Resin** recovery rate is more than 95 % and the mild conditions retain the biological activity of the isolated proteins. Handling is

easy and identical to standard protocols of other manufacturers, therefore there is no need to change established protocols.

Glutathione Agarose Resin

Product	Binding capacity	Size	Cat. no.	
Olutethione Accuracy Desig	Q m c /ml	10 ml	42172.01	
Glutathione Agarose Resin	8 mg/ml	100 ml	42172.02	

**Thrombin from bovine** plasma is suitable for removal of the GST-tag from a recom-

binant fusion protein containing an accessible thrombin recognition sequence.

Product	Specific Activiy	Size	Cat. no.
Thrombin, from bovine plasma, lyophil.	min. 1000 U/mg	250 U	36402.01
		1000 U	36402.02
		5000 U	36402.03

Protein Ark HiFliQ GST FPLC Columns

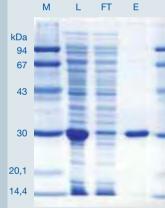
are pre-packed and ready to use for rapid affinity purification of tagged proteins under native conditions. Compatible with all common HPLC and FPLC instruments (including ÄKTA™ FPLCs), and low pressure pumps and syringes using an appropriate adaptor.



#### HiFliQ GST FPLC Columns

Product	Binding capacity	Size	Cat. no.
	10 mg/ml	1 column	42291.01
1 ml HiFliQ GST FPLC Column		5 columns	42292.01
	10	1 column	42293.01
5 ml HiFliQ GST FPLC Column	10 mg/ml	5 columns	42294.01





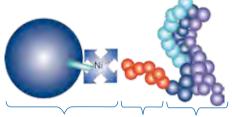
Glutathione Agarose Resin

M = Marker L = Lysate FT = Flowthrough E = Eluate

### B. His-Tag Purification

Recombinant proteins carrying a poly-His are easily purified by immobilized metal affinity chromatography (IMAC). This kind of purification is based on the interaction between superficial protein residues with transition metal cations bound to agarose beads, forming chelated complexes.

#### Principle of His-Tag purification



Ni-NTA agarose beads His-Tag Recombinant protein

**SERVA NTA Agarose Resin** consists of crosslinked agarose derivatized with nitrilotriacetic acid (NTA) and loaded with divalent nickel or cobalt ions. The four metal-binding sites on the chelate enable high protein binding and result in minimal metal leaching, making the resin ideal for purification under reducing conditions. **SERVA Ni-NTA Magnetic Beads** allow the rapid and easy small scale purification of histidine tagged proteins.

Low Pressure NTA Agarose Resins

Product	Binding capacity	Size	Cat. no.	
Ni-NTA	EQ mg (m)	25 ml	42139.01	
INI-INTA	50 mg/ml	100 ml	42139.02	

#### **High Pressure NTA Agarose Resins**

0				
Product	Pressure max.	Binding capacity	Size	Cat. no.
			10 ml	42317.01
Super Ni-NTA	72 psi	30 mg/ml	25 ml	42318.01
			100 ml	42319.01
Super Co-NTA			10 ml	42320.01
	72 psi	30 mg/ml	25 ml	42321.01
			100 ml	42322.01

#### Ni-NTA Magnetic Agarose Beads

Product	Binding capacity	Size	Cat. no.
Ni-NTA	75 mg/ml	2 ml	42179.01
INI-INTA	75 mg/ml	10 ml	42179.02
SERVAMag Rack	-	1 unit	MR-12

High pressure NTA agarose resin is also available as **HiFliQ pre-packed** columns for HPLC/FPLC.

#### HiFliQ/FPLC Columns

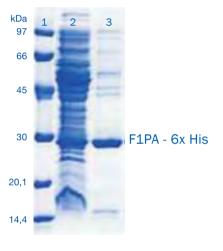
Product	Volume	Binding capacity	Size	Cat. no.
	1 ml		1 column	42283.01
Ni-NTA	1 m	50 75 m c (m)	5 columns	42284.01
N-NTA	5 ml	50 - 75 mg/ml	1 column	42285.01
			5 columns	42286.01
	1 ml		1 column	42287.01
Co-NTA		40 - 50 mg/ml	5 columns	42288.01
CO-NTA		40 - 50 mg/m	1 column	42289.01
	5 ml		5 columns	42290.01



**SERVA IDA Agarose Resins** have iminodiacetic acid (IDA) groups covalently coupled to crosslinked agarose beads and are loaded with a divalent metal. Since IDA has three sides for interaction with metal ions instead of four for NTA, bound proteins can usually be eluted from IDA resins more easily and with lower imidazole concentrations.

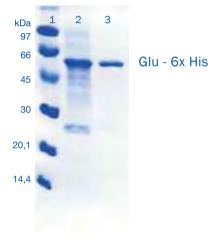
Ni<sup>2+</sup> for higher affinity and lower specificity
 Co<sup>2+</sup> for higher specificity and lower affinity

Purification of Fuculose 1-aldolase (6xHis) with SERVA Ni-IDA HD Agarose Resin



- 1. Low Molecular Weight Markers (LMW)
- 2. F1PA (6xHis) Extract
- 3. Eluate

Purification of Glutarylacylase (6xHis) with SERVA Co-IDA HD Agarose Resin



1. Low Molecular Weight Markers (LMW)

- 2. Glutaryl Acylase (6xHis) Extract
- 3. Eluate

#### Low Pressure IDA Agarose Resins

Туре	Activation grade	Loading capacity Me²+/ml	Size	Cat. no.
Ni-IDA	UD	20 40 umol	25 ml	42141.01
NI-IDA	HD 20 - 40 µmol	100 ml	42141.02	
Co. IDA	HD 20 - 40 µmol	20 40 um al	25 ml	42143.01
Co-IDA		100 ml	42143.02	

#### Ni<sup>2+</sup>-IDA-Metal Chelate Sepharose® Resin for High Pressure Chromatography

Туре	Pressure max.	Binding capacity	Size	Cat. no.
	10	10 mg/ml	25 ml	42315.01
Ni-IDA	42 psi		100 ml	42316.01

The cost-effective **loose resin** is suitable for batch and column purification. SERVA offers empty columns for smaller and larger volumes from 50  $\mu$ l up to 6 ml. For more information please refer to chapter "Empty Columns" at page 11.

The **Ni-Extrachel Agarose Resin** has a polychelator ligand covalently coupled to a highly crosslinked agarose resin and is loaded with nickel ions. The

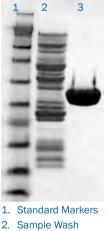
resin works in presence of EDTA, DTT and other chemicals, which result in stripping of the metal ions with standard Ni-NTA or -IDA resins

One-step purification without the need of pre-treatment of samplesHigher resistance against EDTA, DTT, ethanol, etc. as resins of other vendors

#### High Pressure Ni-Extrachel Agarose Resin

Туре	Binding capacity	Size	Cat. no.
Ni Extracted		25 ml	42180.01
Ni-Extrachel	> 80 mg/ml	100 ml	42180.02

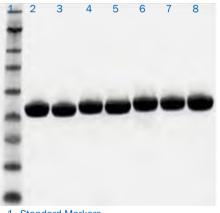
**The Proteus Ni-IMAC kit** is designed for simple, rapid His-tagged recombinant protein purification from a cell lysate under native or denaturing con-



3. Eluate

#### Proteus Ni-IMAC Kits

ditions. Proteus spin columns replace lengthy and expensive chromatographic methods such as FPLC by a rapid one-step purification.



1. Standard Markers
 2. Purified Wild Type Protein
 2. 6 × Durified Mutant Protein

3-8. 6 x Purified Mutant Proteins

Product	Columns	Vivaspin 500 UF Concentrators	Vivaspin 20 UF Concentrators	Buffers	Size	Cat. no.
Mini Kit	24 x 0.23 ml	24	-	yes	1 kit	42269.01
Mini Pack	24 x 0.23 ml	-	-	yes	1 kit	42270.01
Mini Bulk Pack	72 x 0.23 ml	-	-	-	1 kit	42271.01
Mini Sample Kit	4 x 0.23 ml	4	-	yes	1 kit	42268.01
Mini Sample Pack	1 x 0.23 ml	-	-	-	1 kit	42267.01
Midi Kit	8 x 1.6 ml	-	8	yes	1 kit	42272.01
Midi Pack	8 x 1.6 ml	-	-	yes	1 kit	42273.01
Midi Bulk Pack	24 x 1.6 ml	-	-	-	1 kit	42274.01
Buffer Pack	-	-	-	yes	1 kit	42277.01

Protocol for purifying under native and denaturing conditions Different kit formats according to your needs



### C. Antibody Purification (Protein A/G)

Antibody purification is a very important step in obtaining new therapeutic agents. Affinity chromatography is a vital technique in the purification of monoclonal and polyclonal antibodies based on the affinity and specificity of Protein A and Protein G for the Fc region of IgG from a variety of species.

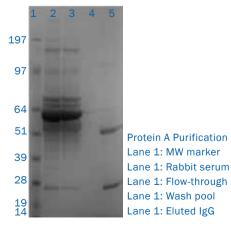
For FPLC applications **Recombinant Protein A and Protein G Sepharose® Resins** are ideal. These resins are designed for simple, one-step and rapid antibody purification from serum, ascites and tissue culture supernatant derived from static cultures and bioreactors. The purified antibody samples can be used in a wide range of laboratory procedures such as 1D or 2D polyacrylamide gel electrophoresis, Western blotting, ELISA etc.

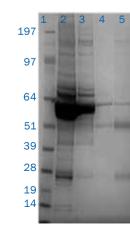
#### Recombinant Protein A/G Sepharose® Resin

Туре	Pressure max.	Binding capacity	Size	Cat. no.
Protein A			1 ml	42309.01
	120 - 140 psi	30 mg/ml	5 ml	42310.01
			25 ml	42311.01
Protein G		20 mg/ml	1 ml	42312.01
			5 ml	42313.01
			25 ml	42314.01

#### HiFliQ Protein A/G FPLC Columns

Туре	Volume	Binding capacity	Size	Cat. no.
Protein A	1 ml		1 column	42295.01
	± 110	30 mg/ml	5 columns	42296.01
	5 ml		1 column	42297.01
			5 columns	42298.01
	4		1 column	42299.01
Protein G	1 ml		5 columns	42300.01
	E ml	20 mg/ml	1 column	42301.01
	5 ml		5 columns	42302.01

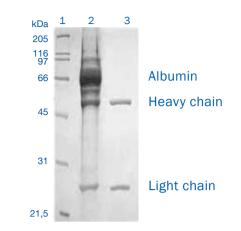




Protein G Purification Lane 1: MW marker Lane 1: Supernatant Lane 1: Flow-through Lane 1: Wash pool Lane 1: Eluted IgG,

Choice of both Protein A & G for complete coverage of antibody subclasses and host species

The innovative **Proteus Protein A and Protein G Mini Kits** combine the quality separation you expect from gravity flow columns with the speed and ease-of-use of spin columns.



# Load Eluate

1. Standard Markers

### Proteus Protein A/G Kits

Product	Columns	Vivaspin 500 UF Concentrators	Vivaspin 20UF Concentrators		Size	Cat. no.
Protein A Mini Kit	16 x 0.23 ml	16	-	yes	1 kit	42256.01
Protein A Mini Bulk Pack Kit	48 x 0.23 ml	-	-	-	1 kit	42257.01
Protein A Mini Sample Kit	2 x 0.23 ml	2	-	yes	1 kit	42255.01
Protein A Mini Sample Pack Kit	1 x 0.23 ml	-	-	-	1 kit	42254.01
Protein A Midi Kit	4 x 1.6 ml	-	4	yes	1 kit	42258.01
Protein A Midi Bulk Pack	12 x 1.6 ml	-	-	-	1 kit	42259.01
Protein G Mini Kit	16 x 0.23 ml	16	-	yes	1 kit	42262.01
Protein G Mini Bulk Pack Kit	48 x 0.23 ml	-	-	-	1 kit	42263.01
Protein G Mini Sample Kit	2 x 0.23 ml	2	-	yes	1 kit	42261.01
Protein G Mini Sample Pack Kit	1 x 0.23 ml	-	-	-	1 kit	42260.01
Protein G Midi Kit	4 x 1.6 ml	-	4	yes	1 kit	42264.01
Protein G Midi Bulk Pack	12 x 1.6 ml	-	-	-	1 kit	42265.01
Protein A and G Starter kit	2 x 0.23 ml, A & G each	-	-	-	1 kit	42266.01
Protein A Buffer Pack	-	-	-	-	1 kit	42275.01
Protein G Buffer Pack	-	-	-	-	1 kit	42276.01



Complete purification in less than 20 (Mini Kits) / 60 (Midi Kits) minutes

#### D. Biotinylated Biomolecules

Due to a superior coupling technology. **SERVA Streptavidin Agarose Resin** provides one of the highest binding capacities available with lower nonspecific binding and less leaching. Recombinant streptavidin is covalently coupled to a highly crosslinked fine beaded agarose for purification of biotinylated biomolecules like proteins, lectins, antibodies, nucleic acids, receptors and ligands. The binding of biotinylated macromolecules is essentially irreversible because of the harsh conditions needed to disrupt the streptavidin-biotin interaction. This feature makes streptavidin-agarose useful in a variety of affinity purification applications.

#### Streptavidin Agarose Resin

Product	Binding capacity	Size	Cat. no.
SERVA Streptavidin Agarose Resin	120 nmol/ml	5 ml 42178.	
SERVA Streptaviolin Agarose Resin	120 111101/111	10 ml	42178.02

### E. Filtration Columns

**Proteus Mini Clarification Spin Col**umns are designed to remove microorganisms, particles and precipitates larger than 0.2 µm pore size from aqueous solutions before HPLC/FPLC separation. The PVDF membrane provides high flow rates and throughput,

low extractables and broad chemical compatibility. The membrane binds far less protein than nylon, cellulose or PES membranes. The columns fit all standard microfuges and allow you to process multiple samples in parallel.

#### Proteus Mini Clarification Spin Columns

Туре	Process	Sample capacity	Pore size	Size	Cat. no.
Mini	Centrifugation	0.65 ml	0.2 µm	100 columns	42225.01

Streptavidin agarose resin with one of the highest specific activity on the market Lowest protein binding PVDF membrane, ideal for HPLC/FPLC sample preparation

### F. Empty Columns

**Mini/Midi/Maxi Columns** for purification by gravity flow. Polypropylene columns containing a polyethylene frit.



#### Mini/Midi/Maxi Columns

Туре	Process	Resin capacity	Sample capacity	Frit pore size	Size	Cat. no.
Mini	Centrifugation	100 - 250 ul	1.5 ml	25 µm	25 columns	42173.01
WITT	Centinugation	100 - 250 μι	1.0 m	25 µm	100 columns	42173.02
Mini Spin	Centrifugation or syringe	50 - 100 µl	0.8 ml	35 µm	25 columns	42176.01
Midi	Crowity flow	0.5 – 2.0 ml	12 ml	00.000	50 columns	42174.01
Maxi	Gravity flow	2 – 6 ml	35 ml	20 µm	50 columns	42175.01

Proteus 1-Step Batch Plus Spin Columns are designed for small scale protein purifications such as those required for expression trials, solubility determination tests, screening, tittering and scouting studies. These innovative columns incorporate a SelfSeal™ membrane technology which retains the resin and sample in the batch incubation chamber. When the column is spun in a benchtop centrifuge at 750 g (for midi spin columns) or at 12 - 14,000 g (for mini spin columns), the pores of the membrane dilate and the filtered eluate is collected in the bottom of the centrifuge tube.

#### 1-Step Batch Spin Columns

Туре	Volume max.	Size	Cat. no.
1-Step Batch Mini Spin Columns	600 ul	40 columns	42237.01
T-Step Batch Mini Spin Columns	600 μι	100 columns	42238.01
1-Step Batch Midi Spin Columns	20 ml	8 columns	42239.01

**Empty FPLC chromatography columns.** Both ends of the FliQ columns have 10.32 UNF threads which fit all common chromatography instruments. Pack your own resin into these columns. The 10.32 Packing Connector is available under 42282.01.

FPL	(:)	olu	mns
	.00	oru	11113

Product	Size	Cat. no.
1 ml FliQ Column		42278.01
5 ml FliQ Column	1 column	42279.01
10 ml FliQ Column		42280.01
20 ml FliQ Column		42281.01

# Gelfiltration

For gelfiltration of protein samples SERVA offers ready-to-use CentriPure and Centri Pure Mini Spin Columns from emp Biotech. The gel matrix Zetadex, a new cross-linked composite dextran matrix, has been proven

- Hydrated, ready-to-use gel filtration columns
- Processing of small and large sample volumes possible
- Available as flow gravity or spin columns
- Fast and easy handling

to be superior for desalting, buffer exchange and removal of small molecular impurities, such as salts, ammonia, dyes, biotin, haptens etc. from antibodies, enzymes and other proteins.

- High yield and high purity
- Minimal effect of buffer and pH on resolution
- Purified biomolecules are directly suited for downstream applications, like SDS PAGE, mass spectrometry, X-ray crystallisation



### CentriPure Gelfiltration Columns

#### Ready-to-use flow gravity columns for rapid purification of proteins from small to large sample volumes

Product	Description	Size	Cat. no.
CentriPure P2 Columns	Size exclusion cut-off: 10 kDa	2 columns	42100.01
	<ul> <li>Sample volume: up to 200 µl</li> <li>Elution volume: 200 to 350 µl</li> </ul>	50 columns	42101.01
CentriPure P5 Columns	Size exclusion cut-off: 10 kDa	2 columns	42102.01
	<ul> <li>Sample volume: up to 0.5 ml</li> <li>Elution volume: 1 ml</li> </ul>		42103.01
CentriPure P10 Columns	Size exclusion cut-off: 10 kDa	2 columns	42104.01
	<ul> <li>Sample volume: up to 1 ml</li> <li>Elution volume: 1.2 to 1.5 ml</li> </ul>	50 columns	42105.01
CentriPure P25 Columns	Size exclusion cut-off: 10 kDa	2 columns	42106.01
	<ul><li>Sample volume: up to 2.5 ml</li><li>Elution volume: 2.7 to 3.5 ml</li></ul>	25 columns	42107.01
CentriPure P50 Columns	Size exclusion cut-off 10 kDa	1 column	42108.01
	<ul> <li>Sample volume: up to 5 ml</li> <li>Elution volume: 6 to 8 ml</li> </ul>	10 columns	42109.01
CentriPure P100 Columns	Size exclusion cut-off: 10 kDa	1 column	42110.01
	<ul> <li>Sample volume: up to 10 ml</li> <li>Elution volume: 12 to 15 ml</li> </ul>	10 columns	42111.01
CentriPure P500 Columns	<ul> <li>Size exclusion cut-off: 10 kDa</li> <li>Sample volume: up to 50 ml</li> <li>Elution volume: 65 to 70 ml</li> </ul>	1 column	42112.01

- Fast and easy handling
- Minimal effect of buffer and pH on resolution

### CentriPure Mini Spin Gelfiltration Columns

#### Ready-to-use spin columns for quick and efficient protein purification from small sample volumes

Product	Description	Size	Cat. no.
CentriPure Mini Spin Columns Desalt Z-50	<ul> <li>Five minutes protocol</li> <li>Purified proteins are eluted into pure,</li> </ul>	4 columns	42113.01
Desait 2-30	deionized water with minimal dilution	25 columns	42114.01
	<ul> <li>Size exclusion cut-off: 25 kDa</li> <li>Sample volume: 10 to 100 µl</li> </ul>	100 columns	42115.01
CentriPure Mini Spin Columns TRIS Z-50	<ul> <li>Five minutes protocol</li> <li>Purified proteins are eluted into 1 mM TRIS.</li> </ul>	4 columns	42124.01
TRI5 2-50	pH 6 with minimal dilution	25 columns	42125.01
	<ul> <li>Size exclusion cut-off: 25 kDa</li> <li>Sample volume: 10 to 100 µl</li> </ul>	100 columns	42126.01
CentriPure Mini Spin Columns PBS Z-50	<ul> <li>Five minutes protocol</li> <li>Purified proteins are eluted into Phosphate</li> </ul>	4 columns	42127.01
PB5 2-50	Buffered Saline (PBS, pH 7) with minimal dilution • Size exclusion cut-off: 25 kDa	25 columns	42128.01
	<ul> <li>Size exclusion cut-off: 25 kDa</li> <li>Sample volume: 10 to 100 μl</li> </ul>	100 columns	42129.01
CentriPure Mini Spin Columns	Five minutes protocol	4 columns	42130.01
Desalt Z-25	<ul> <li>Purified proteins are eluted into pure, deionized water with minimal dilution</li> </ul>	25 columns	42131.01
	Size exclusion cut-off: 5 kDa Sample volume: 10 to 100 µl	100 columns	42132.01
CentriPure Mini Spin Columns TRIS Z-25	<ul> <li>Five minutes protocol</li> <li>Purified proteins are eluted into 1 mM TRIS,</li> </ul>	4 columns	42133.01
	pH 6 with minimal dilution • Size exclusion cut-off: 5 kDa	25 columns	42134.01
	<ul> <li>Sample volume: 10 to 100 µl</li> </ul>	100 columns	42135.01
CentriPure Mini Spin Columns PBS Z-25	<ul> <li>Five minutes protocol</li> <li>Purified proteins are eluted into Phosphate</li> </ul>	4 columns	42136.01
100220	Buffered Saline (PBS, pH 7) with minimal dilution • Size exclusion cut-off: 5 kDa	25 columns	42137.01
	<ul> <li>Sample volume: 10 to 100 µl</li> </ul>	100 columns	42138.01





**Column Preparation** 



#### Sample Application



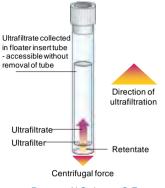
Sample Elution

### High yield and high purity

Purified biomolecules are directly suited for downstream applications, like SDS PAGE, mass spectrometry, X-ray crystallisation

# Ultrafiltration

Ultrafiltration is a fast and simple method for simultaneous concentration of proteins and removal of low molecular weight substances. The unique design of the **Proteus X-Spinner Ultrafiltration Concentrators**  allows not only the efficient purification of membrane proteins, but prevents as well the clogging of the membrane by viscous solutions.



Proteus X-Spinner 2.5

- Cellulose triacetate (CTA) membrane for low protein binding
- Contra-design ensures that membranes do not clog
- Recovery rate > 98 % even with hydrophobic proteins
- Sample volume 0.1 2.5 ml
- Hold-up volume is 25 µl
- Available in five different MWCOs

#### Proteus X-Spinner Ultrafiltration Concentrators

Product	Size	Cat. no.
Diretous V Chinner O.F. E. I/Do MWCO	24 columns	42227.01
Proteus X-Spinner 2.5, 5 kDa MWCO	96 columns	42228.01
Proteus X-Spinner 2.5, 10 kDa MWCO	24 columns	42229.01
Proteus X-Spinner 2.5, 10 kba MWCO	96 columns	42230.01
Proteus X-Spinner 2.5, 20 kDa MWCO		42231.01
Proteus x-Spinner 2.5, 20 kDa MWCO	96 columns	42232.01
Proteus X-Spinner 2.5, 100 kDa MWCO	24 columns	42233.01
Proteus x-Spinner 2.5, 100 kDa MWCO	96 columns	42234.01
Proteus X-Spinner 2.5, 300 kDa MWCO	24 columns	42235.01
Proteus x-spinner 2.5, 500 kDa MWCO	96 columns	42236.01

For test purposes a Proteus X-Spinner 2.5 trial pack can be ordered, containing

# 2 x 5 kDa, 3 x 10 kDa, 2 x 20 kDa, 3 x 100 kDa and 2 x 300 kDa columns:

Product	Size	Cat. no.
Proteus X-Spinner 2.5 Trial Columns, assorted MWCOs	12 columns	42226.01

Ideal for membrane proteins and viscous samples

De-proteinization of blood and serum samples



# **Protein Purification Kits**

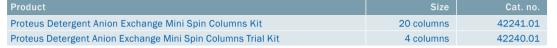
As special applications in protein purification SERVA offers kits for

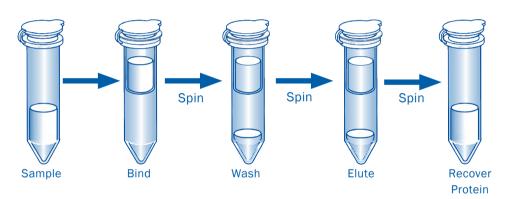
- Endotoxin removal
- Detergent removal

The **Proteus Detergent Anion Exchange Mini Spin Column Kit** removes excess detergents and concentrates proteins in only 10 minutes.

- Complete detergent exchange/removal
- Binding capacity is 2 mg
- Minimum elution volume is 50 µl

#### Proteus Detergent Anion Exchange Mini Spin Column Kit



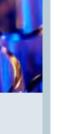


The **Proteus NoEndo™** spin column kits offer a standardised method for high grade clearance of endotoxin from recombinant proteins, antibodies and viral vectors.

- Endotoxin-free preparation in less than 1 hour (for M, S, HC)
- SelfSeal<sup>™</sup> membrane technology to prevent leaking into the collection tube (µ, M)
- FlowGo<sup>™</sup> membrane technology for sample movement regulation (S, HC)
- µ and M-Kits include loose resin and spin columns
- S and HC kits include pre-packed spin columns

#### **Specifications:**

Spin columns	NoEndo μ	NoEndo M	No Endo S	NoEndo HC
Binding capacity per column	300 - 500 EU	3,000 EU	30,000 EU	1,000,000 EU
Binding capacity per ml	500 - 800 EU	300 EU	1,500 EU	30,000 EU
Minimum endotoxin levels tested post-column	<0.03 EU/ml	<0.03 EU/ml	<0.05 EU/ml	<0.05 EU/mI
Endotoxin clearance after 1 pass	-	-	3 log reduction	3 log reduction
Endotoxin clearance after 2 passes	-	-	4 log reduction	4 log reduction
Endotoxin clearance after 1 hour incubation	3 log reduction	2 log reduction	-	-
Endotoxin clearance after 3 hour incubation	4 log reduction	3 log reduction	-	-
Maximum sample load volume	0.6 ml	20 ml	20 ml	20 ml
NoEndo resin bed volume	0.01 - 0.1 ml loose	0.25 ml loose	1 ml pre-packed	1.7 ml pre-packed



#### Proteus NoEndo<sup>™</sup> Spin Columns

Product	Size	Cat. no.
Proteus NoEndou (Micro) Column Kit	2 columns	42242.01
	24 columns	42246.01
Proteus NoEndoµ (Micro) Column Kit	100 columns	42250.01
Proteus NoEndoM (Mini) Column Kit	2 columns	42243.01
	12 columns	42247.01
	48 columns	42251.01
	2 columns	42244.01
Proteus NoEndoS (Standard) Column Kit	12 columns	42248.01
	48 columns	42252.01
	2 columns	42245.01
Proteus NoEndoHC (High Capacity) Column Kit	12 columns	42249.01
	48 columns	42253.01



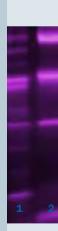
For studying the roles of kinases and phosphatases in signaling pathways, choosing the right phosphatase inhibitor is very important. To ensure immediate inhibition of all phosphatase acitivities during cell lysis, use SERVA's fine-tuned and also broadly effective inhibitor mixes.

To isolate proteins in their native phosphorylation state

#### One vial is equivalent to 1 ml of 100x concentrate

#### Phosphatase Inhibitor Mixes

Product	Application	Size	Cat. no.
Phosphatase Inhibitor	Contains 5 water soluble phosphatase inhibitors. Inhib-	1 vial	39050.01
Mix I, powder	its acid and alkaline phosphatases, protein phospha- tases 2A, 2B and 2C, phosphoprotein phosphatases,	5 vials	39050.02
	and protein-tyrosine phosphatases.		39050.03
Phosphatase Inhibitor	bitor Contains 7 phosphatase inhibitors, dissolved in water. Inhibits acid and alkaline phosphatases, protein phos- phatases 2A, 2B and 2C, phosphoprotein phosphatases,	1 vial	39055.01
Mix II, solution		5 vials	39055.02
	protein-tyrosine phosphatases, and serine/threonine phosphatases.	10 vials	39055.03



Protease (PIMG) r protection of proteoly by Neuter (lane 5: and 3: F lane 2 a plus NP

# Protease Inhibitor Mixes

During the preparation of cell extracts proteases are inevitably released from bacteria, yeast, tissue or cell cultures. To achieve highest possible recoveries of native proteins the addition of inhibitors of these enzymes is essential. With SERVA's application-optimized inhibitor mixes there is no need for tedious testing of self-made compositions of various protease inhibitors. Inhibitor mixes as powder are more effective in protease inhibition than other formulations. No splitting of tablets at lower volumes is necessary.

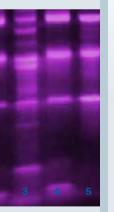
- Efficient protection of proteins against proteolytic degradation
- One vial is equivalent to 1 ml of 100x concentrate
- DMSO for resuspension included for all non-water soluble mixtures

### Protease Inhibitor Mixes

Reduced health risk in handling of protease and phosphatase inhibitors
Also available: over 20 individual protease inhibitors for customized applications

Protease Inhibitor	For general applications, and where the use of organic	1 vial	39101.01
Mix G	solvents should be avoided. Contains 5 water soluble protease inhibitors. Inhibits	5 vials	39101.02
	cysteine, serine- and metallo-proteases.	10 vials	39101.03
Protease Inhibitor	For use with extracts from mammalian tissue.	1 vial	39102.01
Mix M	Contains 6 protease inhibitors. Inhibits aspartate-, cysteine-, serine-, and metallo-proteases as well as	5 vials	39102.02
	aminopeptidases.	10 vials	39102.03
Protease Inhibitor	For use with plant extract.	1 vial	39103.01
Mix P	Contains 6 protease inhibitors. Inhibits aspartate-, cysteine-, serine-, and metallo-proteases as well as	5 vials	39103.02
	aminopeptidases.	10 vials	39103.03
Protease Inhibitor	For use with fungus and yeast extracts.	1 vial	39104.01
Mix FY	Contains 4 protease inhibitors. Inhibits aspartate-, cysteine-, serine-, and metallo-proteases.	5 vials	39104.02
		10 vials	39104.03
Protease Inhibitor	For use with bacterial extracts.	1 vial	39105.01
Mix B	Contains 5 protease inhibitors. Inhibits aspartate-, cysteine-, serine-, and metallo-proteases as well as	5 vials	39105.02
	aminopeptidases	10 vials	39105.03
Protease Inhibitor	For purification of polyHis-tagged proteins.	1 vial	39106.01
Mix HP	Contains 4 water soluble protease inhibitors. Inhibits cysteine- and serine-proteases.	5 vials	39106.02
		10 vials	39106.03
Protease Inhibitor	For purification of polyHis-tagged proteins.	1 vial	39107.01
Mix HP Plus	Contains 6 protease inhibitors. Inhibits aspartate-, cys- teine- and serine-proteases as well as aminopeptidases,	5 vials	39107.02
	Thermolysin and other microbial metallo-proteases.	10 vials	39107.03





e Inhibitor Mix G mediated efficient on ins against tic degradation ral Protease (NP) Control, Iane 1 roteins plus NP; nd 4: Proteins plus PIMG)

# **Protein Quantification**

Accurate determination of protein concentration is essential in the protein analysis workflow. Although there are a wide variety of protein assays available, none of the assays can be used without first considering their suitability for the application. Each assay has its own advantages and limitations and often it is necessary to obtain more than one type of protein assay for research applications.

#### Standard assays for protein quantification

- Bradford reagent, 5x concentrate
- Suitable for micro (1 25  $\mu$ g protein/ml) and standard (0.1 1mg protein/ml) assays
- Lowry Assay Kit
  - Contains ready-to-use reagents including protein standard solution

Improved assays compatible with detergents and reducing agents

- BCA Assay Kit
- Assay based on bicinchoninic acid method
- Compatible with many detergents
- · Less binding variation between different proteins than Bradford assay
- SingleQuant Assay Kit
  - Based on the method of Popov\*
  - No interference with detergents and reducing agents
  - Detection of 2 µg to 1,400 µg per sample
- SERVA Purple Protein Quantification Assay
  - A non-toxic, eco-friendly fluorescent dye assay
  - Compatible with many detergents and reducing agents
  - Accurate staining of glyco-, phospho-, hydrophobic proteins and peptides
  - Single tube (200 assays), 96- or 384-well-format for HTS (up to 10.000 assays)
  - Detection limit of 100 ng/ml for peptides and 40 ng/ml for proteins

Product	Description	Size	Cat. no.
Bradford reagent,	For protein quantification after Bradford	50 ml	39222.01
5x concentrate		200 ml	39222.02
		500 ml	39222.03
Lowry Assay Kit	For protein quantification after Lowry	250 tests	39236.01
BCA Protein Assay Micro Kit	Based on bicinchoninic acid method	480 tests	39229.01
BCA Protein Assay Macro Kit	Based on bicinchoninic acid method	250 tests	39228.01
		500 tests	39228.02
SingleQuant Assay Kit	Record on the energy method of Denoy	200 tests	39226.01
	Based on the assay method of Popov	600 tests	39226.02
SERVA Purple Protein Quantification Assay	Based on fluorescent dye SERVA Purple	10 ml	39235.01

\* Popov, N. et. Al. (1975) Acta Biol. Med. Ger. 34(9), 1441-1446

Fast, reliable and reproducible measurement of protein concentrations

Improved assays for less sample buffer-mediated restrictions

# **Enzymes Used in Sample Preparation**

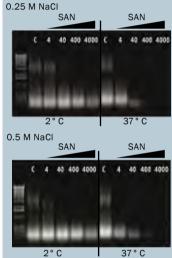
### Salt Active Nuclease

Engineered highly active non-specific endonuclease that tolerates reaction conditions with high salt concentrations.

Salt is an important component of various purifications schemes. The presence of salt can minimize aggregation, increase target solubility and improve target yield. High salt enables contaminating DNA to dissociate from associated proteins and become available for degradation. The optimal activity at high salinity, the resistance to non-ionic detergents and the easy inactivation and separation of the enzyme from other proteins make Salt Active Nuclease the superior choice for DNA digestion in the protein purification workflow.

Only nuclease with an optimum activity in 0.5 M NaCl Active at low temperatures and high pH range

- Easily inactivated by denaturing reagents
- Due to high pl of 9.6 easily removed by cationic exchange columns
- SAN High Quality for removal of nucleic acids in manufacturing and bioprocessing of therapeutic proteins, viruses and similar compounds
- SAN High Quality ELISA for demonstration of removal



2 0	•	01	•
Incubation of E. coli	lysate w	vith in	creasing
units of Salt Active N	luclease	e for 3	80 min

Product	Size	Cat. no.
Salt Active Nuclease	5 000 U	18541.01

### TEV Protease, recombinant

Highly site-specific cysteine protease for the very efficient removal of fusion tags from recombinant proteins

Genetically modified to increase activity and resistance to autolysis

Contains a N-terminal polyhistidine tag for easy removal from the cleavage reaction by affinity chromatography

Supplied with 1 ml 20x TEV Reaction Buffer and 100 mM DTT

Product	Size	Cat. no.
TEV Protease, recombinant, 1 U/µI, solution	1000 U	36403.01

Effective reduction of viscosity caused by nucleic acids for shorter processing time and increased yield of proteins

TEV Protease with increased activity for very efficient removal of fusion tags



# SERVA ICPL™ Kit

The ICPL<sup>™</sup> technology combines the power of Isotope Coded Protein Labeling with an unmatched dynamic range for protein identification and quantification due to its potential combination with intact protein fractionation steps. Whereas by other methods the labeling step is only performed after the proteolytic digestion, that is on the peptide level, with the ICPL<sup>™</sup> kit the labeling step is made already on the protein level and includes all free amino acid groups. Therefore the analytical depth of a proteome analysis is improved significantly. While the ICPL<sup>TM</sup> kit, the ICPL<sup>TM</sup> Triplex and Quadruplex kit is designed to analyse two, three or four proteomes ( $2 \times 6$ ,  $3 \times 6$ and  $4 \times 6$  reactions, respectively), the ICPL Quadruplex Plus kit includes MS approved endoproteinase Trypsin NB as well as Glu-C for achieving the highest sequence coverage ( $4 \times 6$  reactions). A software to analyse MS data generated by ICPL-based technique, is available free of charge.



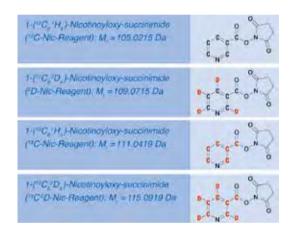
DGE LC. FFE

FFE

1D no

H.LC

640



For the stable labeling of intact proteins  $1\cdot ({}^{12}C_6 \, {}^{11}H_4)$ -nicotinoyloxy-succinimide,  $1\cdot ({}^{12}C_6 \, {}^{11}H_4)$ -nicotinoyloxy-succinimide,  $1\cdot ({}^{12}C_6 \, {}^{2D}4)$ -nicotinoyloxy-succinimide and  $1\cdot ({}^{13}C_6 \, {}^{2}D_4)$ -nicotinoyloxy-succinimide is used.

#### References:

- 1. Schmidt, A., Kellermann, J. and Lottspeich, F. (2005), Proteomics 5, 4-15
- 2. Brunner, A., Keidel, E., Dosch, D., Kellermann, J. and Lottspeich, F. (2010) Proteomics 10, 315-326

	SER		39230
Product		Size	Cat. no.
SERVA ICPL™ Kit		1 kit	39230.01
SERVA ICPL™ Triplex Kit		1 kit	39231.01
SERVA ICPL™ Quadruplex Kit		1 kit	39232.02
SERVA ICPL™ Quadruplex Plus Kit		1 kit	39233.01

Quantitative analysis of two, three or four proteomes in mass spectrometry

Quadruplex Plus version for highest sequence coverage

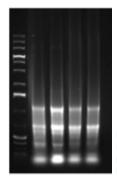
Analysis of posttranslational modifications and of isoforms

# Nucleic Acid Sample Preparation Buffers & Enzymes

## BlueZol

For the rapid isolation of total RNA from cells and tissues of human, animal, plant, yeast or bacterial origin.

- Suitable for the simultaneous isolation of RNA, DNA and protein from one sample
- Purified RNA is ideal for any downstream applicationssuch as RT-PCR, in vitro translation, Northern Blotting,RNase protection assays or dot blot hybridization
- Purified DNA can be used for PCR and Southern Blotting and the proteins for Western Blotting



Lane 1. kb Marker Lane 2 - 5: Total RNA preparations from rat

Product	Size	Cat. no.
BlueZol lysis reagent for cells and tissues	100 ml	39808.01

### Lysozym from chicken egg white

Enzyme, which hydrolyzes the  $\beta$  1  $\rightarrow$  4 linkages of the murein between N-acetylmuramic acid and N-acetyl-D-glucosamin and degrades the heteroglycan chain to disaccharides. Crystallized, salt free, albumin free

Lysis of bacterial cells for extraction of nucleic acids

Used for preparation of spheroplasts

Product	Size	Cat. no.
Lysozyme from chicken egg white min. 15 000 units/mg cryst.	2.5 g	28263.01
	10 g	28263.02

## Zymolyase<sup>®</sup> from Arthrobacter luteus

Enzyme complex with strong lytic activity against living yeast cell walls. The essential enzyme activity is  $\beta$ -1,3-glucan laminaripentaohydrolase, which hydrolyzes linear glucose polymers with  $\beta$ -1,3-linkages. Contained main side activities are  $\beta$ -1,3-glucanase, protease, and mannanase.

Used for preparation of protoplasts or spheroplasts of various yeast strains

Product	Size	Cat. no.
Zymolyase® von Arthrobacter luteus, min. 20 U/mg lyophil.	100 mg	33759.01
	500 mg	33759.02
	1 g	33759.03
Zymolyase® von Arthrobacter luteus, min. 100 U/mg lyophil.	100 mg	33760.01
	500 mg	33760.02



### CTAB DNA Extraction Buffer

Ready-to-use buffer with the non-ionic detergent cetyltrimethylammonium-bromide (CTAB) for removal of polysaccharides contamination during isolation of DNA from plants. By adjustment of salt concentration in lysates containing CTAB, polysaccharides and DNA can be differentially precipitated.

Product	Size	Cat. no.
CTAB DNA Extraction Buffer molecular biology grade	500 ml	39809.01

### Proteinase K from Tritirachium album

Subtilisin-related serine protease with a very high specific activity and a broad spectrum of action. It is widely used for digestion of proteins, including DNases and RNases during nucleic acid preparations without compromising the integrity of the isolated DNA or RNA.

- Free of endonucleases, exonucleases, and ribonucleases
- Recombinant enzyme with very low DNA content
  - Molecular biology grade: ≤10 pg/mg enzyme
  - NGS grade: ≤0.1 pg/mg enzyme

NGS grade with 2.5-fold increased solubility and increased specific activity

Product	Size	Cat. no.
Proteinase K from Tritirachium album, min. 30 mAnson-U/mg	25 mg	33752.01
	100 mg	33752.02
	500 mg	33753.03
Proteinase K from Tritirachium album solution, 20 mg solid/ml, $\geq$ 600 mAnson-U/ml	1 ml	33755.01
	5 ml	33755.02
	10 ml	33755.03
Proteinase K, recombinant, min. 30 mAnson-U/mg, lyophil., molecular biology grade	100 mg	33756.02
	500 mg	33756.03
Proteinase K, recombinant, min. 35 mAnson-U/mg, lyophil., NGS grade	25 mg	33757.03
	100 mg	33757.02
	500 mg	33757.03

### Pronase E from Streptomyces griseus

Non-specific protease with neutral protease, chymotrypsin, trypsin, carboxypeptidase, aminopeptidase, and neutral and alkaline phosphatase activity. Pronase E has a broad specificity, breaking down proteins into their individual amino acids. Suitable for the extraction of phage DNA and isolation of plasmid DNA.

Very stable in the pH range 6.0. – 9.0

Can be completely inactivated by heating at greater than 80 °C for 15 - 20 minutes

Product	Size	Cat. no.
Pronase E from Streptomyces griseus, min. 5.0 DMC-U/mg, lyophil.	250 mg	33635.01
	1 g	33635.02
	5 g	33635.03



# **Reagents and Solutions**

SERVA's molecular biology grade reagents are guaranteed DNAse/RNase free and allow the efficient isolation of pure and intact nucleic acids.



Product	Size	Cat. no.
Ammonium acetate, molecular biology grade	500 g	39750.01
	250 ml	39751.01
Ammonium acetate solution, 7.5 M, molecular biology grade	11	39751.02
Ammonium chloride, molecular biology grade	500 g	39752.01
Octultzing athulan manium abzamida (OTAD) azust anusa	100 g	16530.04
Cetyltrimethylammonium • bromide (CTAB) cryst., pure	500 g	16530.02
Chloroform, molecular biology grade	250 ml	39533.01
Chloroform:Isoamyl alcohol 24:1, molecular biology grade	500 ml	39554.02
	250 ml	39556.01
Ethanol undenatured absolute, molecular biology grade	11	39556.02
	2.51	39556.03
Ethylenediamine tetraacetic acid•Na2-salt (EDTA), molecular biology grade	250 g	39760.01
0.5 M Ethylenediamine tetraacetic acid• Na2-salt solution (EDTA),	100 ml	39761.01
molecular biology grade	500 ml	39761.02
Olyaptan from overtar, colution 20 md (ml. malcoular biology drade	1 ml	39766.01
Glycogen from oyster, solution 20 mg/ml, molecular biology grade	10x 1 ml	39766.02
	100 g	39558.01
Guanidine • HCI, molecular biology grade	500 g	39558.02
	1 kg	24205.02
Quanidina thiasyanata, malagular hialagu grada	250 g	39577.01
Guanidine thiocyanate, molecular biology grade	500 g	39577.02
locomyl clockel, melecular biology grade	250 ml	39557.01
Isoamyl alcohol, molecular biology grade	11	39557.02
	250 ml	39559.01
leonronanol, molecular biology grade	11	39559.02
Isopropanol, molecular biology grade	500 ml	39772.02
	500 g	39773.02
Phenol, analytical grade, Ph. Eur., USP	500 g	32046.02
Polyethylene glycol 6000, molecular biology grade	500 g	39778.01
Potassium acetate, molecular biology grade	500 g	39567.02
Potassium chloride, molecular biology grade	500 g	39768.01
20 % CDC colution molecular history grade	100 ml	39575.01
20 % SDS solution, molecular biology grade	11	39575.02
Sodium acetate, molecular biology grade	500 g	39571.01
3M sodium acetate buffer solution pH 5.2, molecular biology grade	250 ml	39572.01
Sodium chlorido, molocular biology grada	250 g	39781.01
Sodium chloride, molecular biology grade	1 kg	39781.02
Di-Sodium hydrogen phosphate•2H20, molecular biology grade	1 kg	39783.02
TE Puffor (100v) pH 8.0 molecular biology grade	100 ml	39799.01
TE Buffer (100x) pH 8.0, molecular biology grade	500 ml	39799.02
Tris(hydroxymethyl)aminomethane, molecular biology grade	500 g	37186.02
	1 kg	37186.03
	2.5 kg	37186.04
Tris Buffer pH 7.5, 1 M solution, molecular biology grade	1 L	39791.01
Tris Buffer pH 8.0, 1 M solution, molecular biology grade	1 L	39792.01



# serva worldwide WWW.Serva.de



