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Mobicols - Plastic Columns

Tools for diverse Lab Applications

SEPARATION

ISOLATION

TREATMENT

PURIFICATION

Mo Bi Tec®
MOLECULAR BIOTECHNOLOGY

Contents

Mobicols are versatile columns which can be used for many lab tasks. They are compatible with sample sizes from very small to very large volumes, and can handle small or large wash buffer volumes **(Chapter I).**

Mobicols can be used to make your own spin/affinity columns with the matrix of your choice **(Chapter II).**

Used as a Mini Column they feature Luer-lock connections on top and bottom. Using the Luer-lock adapter, a syringe can be connected to the top of the Mobicol as a reservoir for sample or buffer application. To enable a connection to larger reservoirs, a Luer-adapter is available to attach tubing to the top cap. As Spin Columns, Mobicols are placed in a 1.5 ml or 2 ml microtube and can be centrifuged in a microcentrifuge.
Spin Columns can be used for the purification of biomolecules from small contaminants (e.g. desalting) **(Chapter III).**

Applying Mini Columns, you can take advantage of highly active immobilized enzymes, packed into small Compact Reaction Columns (CRC) **(Chapter IV).** With more than 20 years of experience in immobilizing native proteins and active enzymes, MoBiTec offers a custom immobilization service at very attractive conditions.

Chapter I

Mobicol – A Practical Tool for Every Lab

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Mobicol ”F“

Mobicols are 700 µl columns with particular handling advantages:

- Mobicols are compatible with laboratory standards
- Mobicols can be centrifuged in microcentrifuges
- Mobicols have a Luer-lock adapter
- Smallest und largest volumes can be treated easily

Luer-lock cap

closing cap

35 µm filter

plug

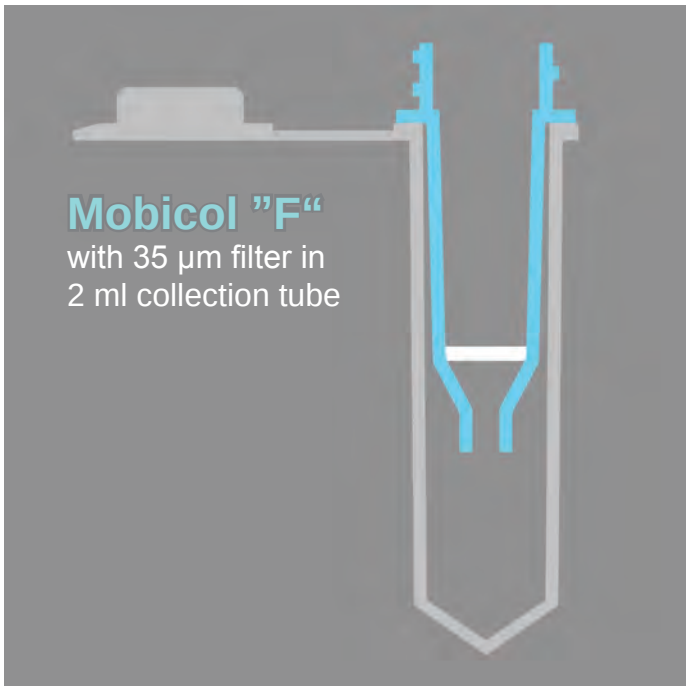
Syringe as reservoir connected to Luer-lock cap of Mobicol

Adapter M3001 tubing to Mobicol

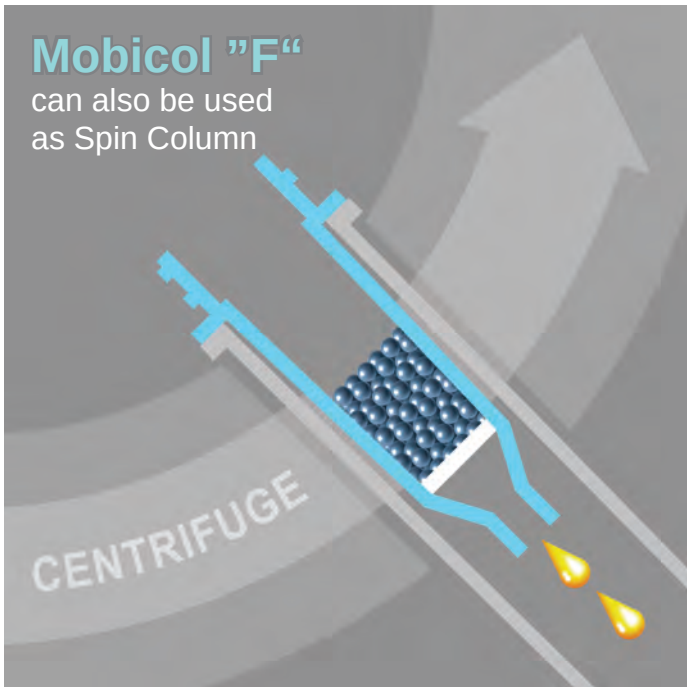
Filter of 35 µm pore size is inserted. A separately available upper filter (Order No. M523515) conveniently caps the resin bed.

Mobicol ”F“ can be closed tightly with a screw-on cap and a snap-off plug at the outlet.

Mobicol ”F“ can be autoclaved at 110 °C for 10 minutes.



Mobicol ”F“
with 35 µm filter in
2 ml collection tube



Mobicol ”F“
can also be used
as Spin Column

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Mobicol ”F“ M105035F

Mobicol ”F“ M105035F with fixed snap-off plug for the outlet is supplied with 2 caps: a Luer-lock cap and a screw cap. The Mobicol ”F“ can be closed tightly with screw cap and plug. A filter of 35 µm pore size is inserted; an additional upper filter (35 µm) is available.

Mobicol ”F“ is approved

- Hundreds of satisfied customers are using Mobicols already for many years
- Many of referenced applications

Mobicol ”F“ is versatile

- Can be used in flow-through mode or as Spin Column
- Useful for size exclusion or affinity purification of compounds like nucleic acids and proteins (native or tagged)
- Compatible with small and large sample volumes
- Upper filters of 35 µm pore size are separately available
- Screw cap with rubber seal
- Mobicol can be filled and stored or shipped without leakage
- Reverse side of snap-off plug can be used for resealing

Mobicol ”F“ is compatible with lab standards

- Can be centrifuged in standard microcentrifuge
- Luer adapters are available for inlet and outlet tubing
- Luer-lock cap is compatible to Luer syringes
- Autoclavable with inserted filter at 110 °C for 10 min

Applications

Use it with affinity matrix for:

- **Purification of tagged proteins**
- **Purification of peptides**
- **Purification of native proteins**
  using immobilized IgG, NTP-binding proteins (e.g. kinases), amino acid binding proteins, β-lactamase, Igase, peroxidase, EPO (erythropoetin), β-galactosidase
- **Fractionation and purification of antibodies**
  using protein A/G, immobilized epitopes, peptide matrix
- **Purification of glycoproteins and carbohydrates**
- **Purification of fatty acids and fatty acid binding proteins**
- **Whole mount embryo *in situ* hybridization**

Order information

Order No.	Description	Amount
M105035F	Mobicol ”F“ with fixed outlet plug, inserted 35 µm filter and 2 different screw caps	50
M1050	Mobicol ”F“ with fixed outlet plug and 2 different screw caps, without filters	50
M1053	Mobicol ”F“ with fixed outlet plug and screw cap, without filter	50
M2210	Filter (large) 10 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M523515	Filter (large) 35 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M2190	Filter (small) 90 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50

For column accessories see page 7.

Also available prefilled with immobilized enzymes:
Immobilized proteases, nucleases, alk. phosphatase, β-galactosidase, or a compound of your choice on request (see chapter IV).

Mobicol ”Classic“

Use for Affinity Chromatography (M1002)

with inserted filters (large)

Mobicol ”Classic“

Use as Spin Column (M1003)

with inserted filters (small)

The Mobicol “Classic” is supplied with two screw caps (Luer-lock cap and screw cap) and a plug for the outlet. Small and large filters with different diameters (10 µm, 35 µm and 90 µm) are separately available, so you can use the filter of your choice. With this features the Mobicol “Classic” can be used for a variety of applications.

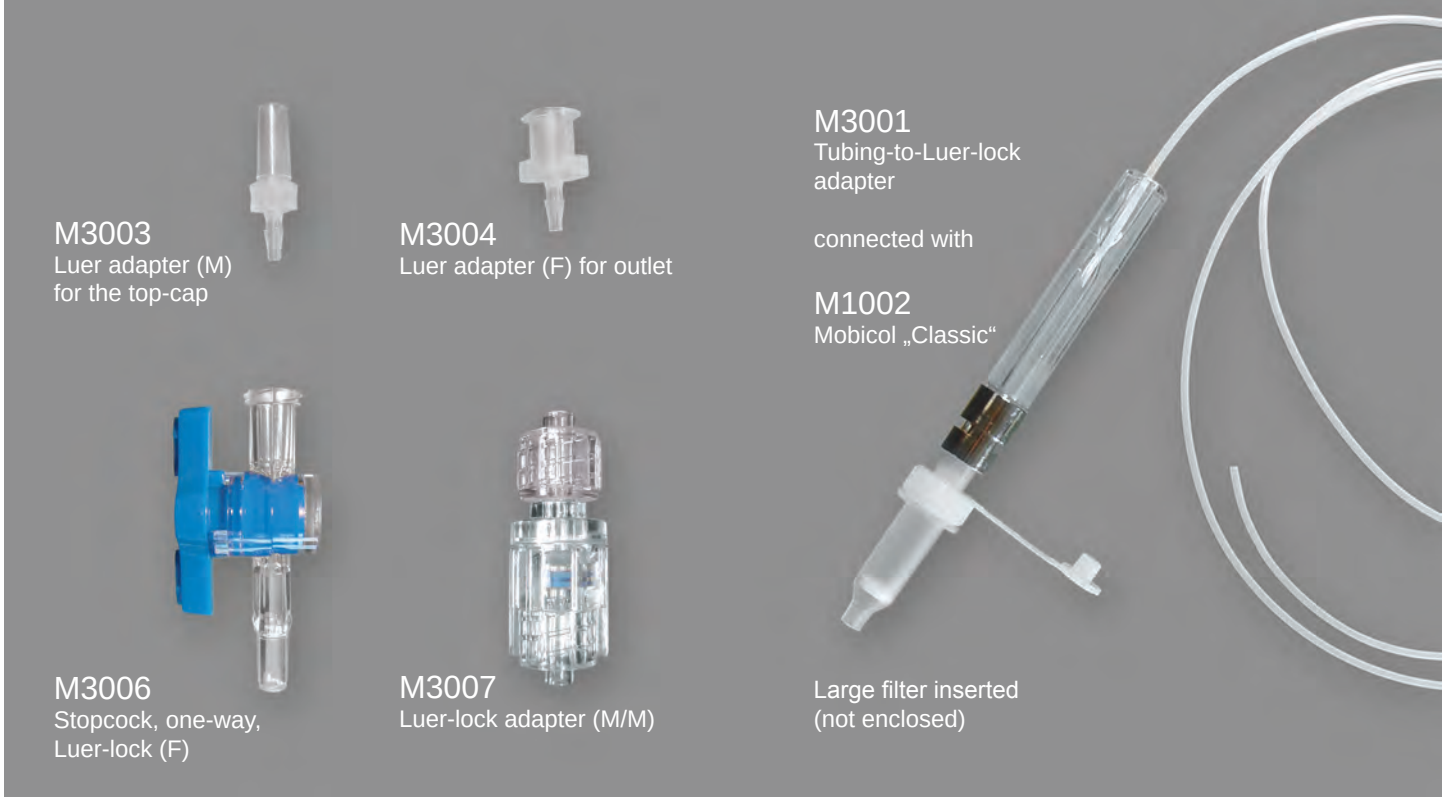
Filters and insertion tools

(small) (large)

Mobicol ”Classic“

Order No.	Description	Amount
M1002	Mobicol ”Classic“ with 2 different screw caps, without filters	50
M1003	Mobicol ”Classic“ with 1 screw cap, without filters	50
M2110	Filter (small) 10 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M513515	Filter (small) 35 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M2190	Filter (small) 90 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M2210	Filter (large) 10 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M523515	Filter (large) 35 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50
M2290	Filter (large) 90 µm pore size, for Mobicol M1002, M1003, M1050 & M1053	50

Accessories for Affinity Chromatography Columns



The polypropylene Luer adapter (M) (M3003) for tubing connection to the inlet of the column allows a flow-trough mode at low pressure, whereas the Luer adapter (F) (M3004) is suitable for a tubing connection to the outlet of the column. Furthermore the Luer adapter (M/M) (M3007) facilitates the connection of the inlet to a Luer-lock system.

The Tubing-to-Luer-lock adapter (M3001) enables a safe connection from a pump to the inlet of a Mobicol or a 2.5 ml column.

The one-way stopcock, Luer-lock (F) (M3006) is a convenient tool for controlling flow-through at the in- or outlet.

Column Accessories

Order No.	Description	Amount
M3001	Tubing-to-Luer-lock adapter (material outside: metal; inside: glass and Teflon; with 1 m Teflon tubing) for Mobicols or laboratory columns	1
M3002	Luer adapter, (M+F) 10 each	20
M3003	Luer adapter for the top-cap (M)	20
M3004	Luer adapter for outlet (F)	20
M3007	Luer-lock adapter (M/M)	1
M3005	Bottom plugs for Mobicols	50
M3006	Stopcocks, one-way, Luer-lock (F)	4
M3009	Luer-lock caps	50

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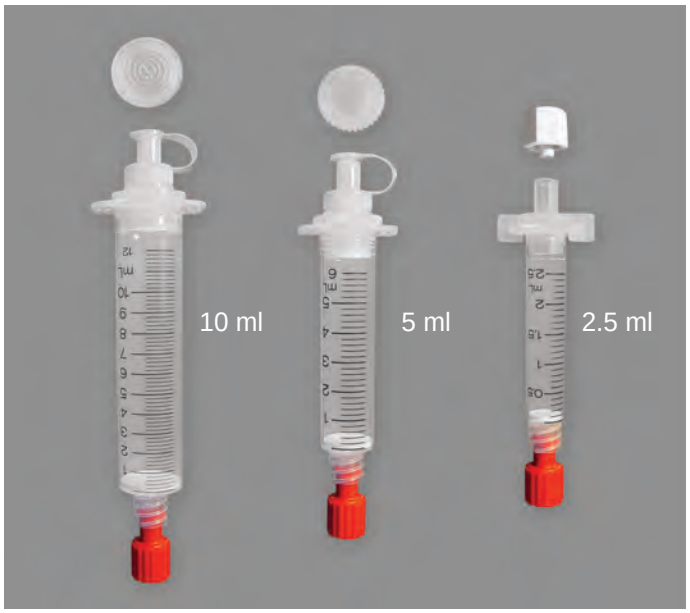
Related Products

For volumes larger than 1 ml (Mobicols), 2.5 ml, 5 ml and 10 ml columns are available with filters inserted. These syringe based columns have Luer-lock adapters at their in- and outlet so that they have the same handling advantages as Mobicols.



Laboratory columns

Order No.	Description	Amount
S10121	2.5 ml columns (10 µm filter pore size)	20
S1012	2.5 ml columns (35 µm filter pore size)	20
S10129	2.5 ml columns (90 µm filter pore size)	20
S10131	5 ml columns (10 µm filter pore size)	20
S1013	5 ml columns (35 µm filter pore size)	20
S10139	5 ml columns (90 µm filter pore size)	20
S10141	10 ml columns (10 µm filter pore size)	20
S1014	10 ml columns (35 µm filter pore size)	20
S10149	10 ml columns (90 µm filter pore size)	20
S1210	Upper filter 10 µm for 2.5 ml columns	20
S1235	Upper filter 35 µm (thickness 1.5 mm) for 2.5 ml columns	20
S123532	Upper filter 35 µm (thickness 3.2 mm) for 2.5 ml columns	20
S1290	Upper filter 90 µm for 2.5 ml columns	20
S1310	Upper filter 10 µm for 5 ml columns	20
S1335	Upper filter 35 µm (thickness 1.5 mm) for 5 ml columns	20
S133532	Upper filter 35 µm (thickness 3.2 mm) for 5 ml columns	20
S1390	Upper filter 90 µm for 5 ml columns	20
S1410	Upper filter 10 µm for 10 ml columns	20
S143532	Upper filter 35 µm (thickness 3.2 mm) for 10 ml columns	20
S1490	Upper filter 90 µm for 10 ml columns	20

Column sets

Order No.	Description	Amount
S10011	Set: Mobicol ”F“ M1050; 2.5 ml ; 5 ml and 10 ml columns (10 µm pore size filter)	3 each
S1001	Set: Mobicol ”F“ M1050; 2.5 ml ; 5 ml and 10 ml columns (35 µm pore size filter)	3 each
S10019	Set: Mobicol ”F“ M1050; 2.5 ml ; 5 ml and 10 ml columns (90 µm pore size filter)	3 each
S10031	Set upper filters 10 µm pore size for S10011	1 set
S1003	Set upper filters 35 µm pore size for S1001	1 set
S10039	Set upper filters 90 µm pore size for S10019	1 set

Chapter II

Spin Columns for your own Matrices or other Compounds

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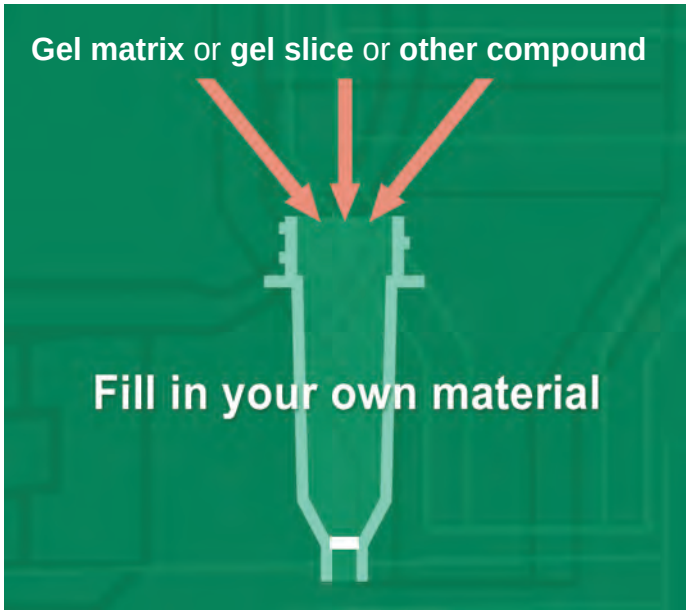
Advantages

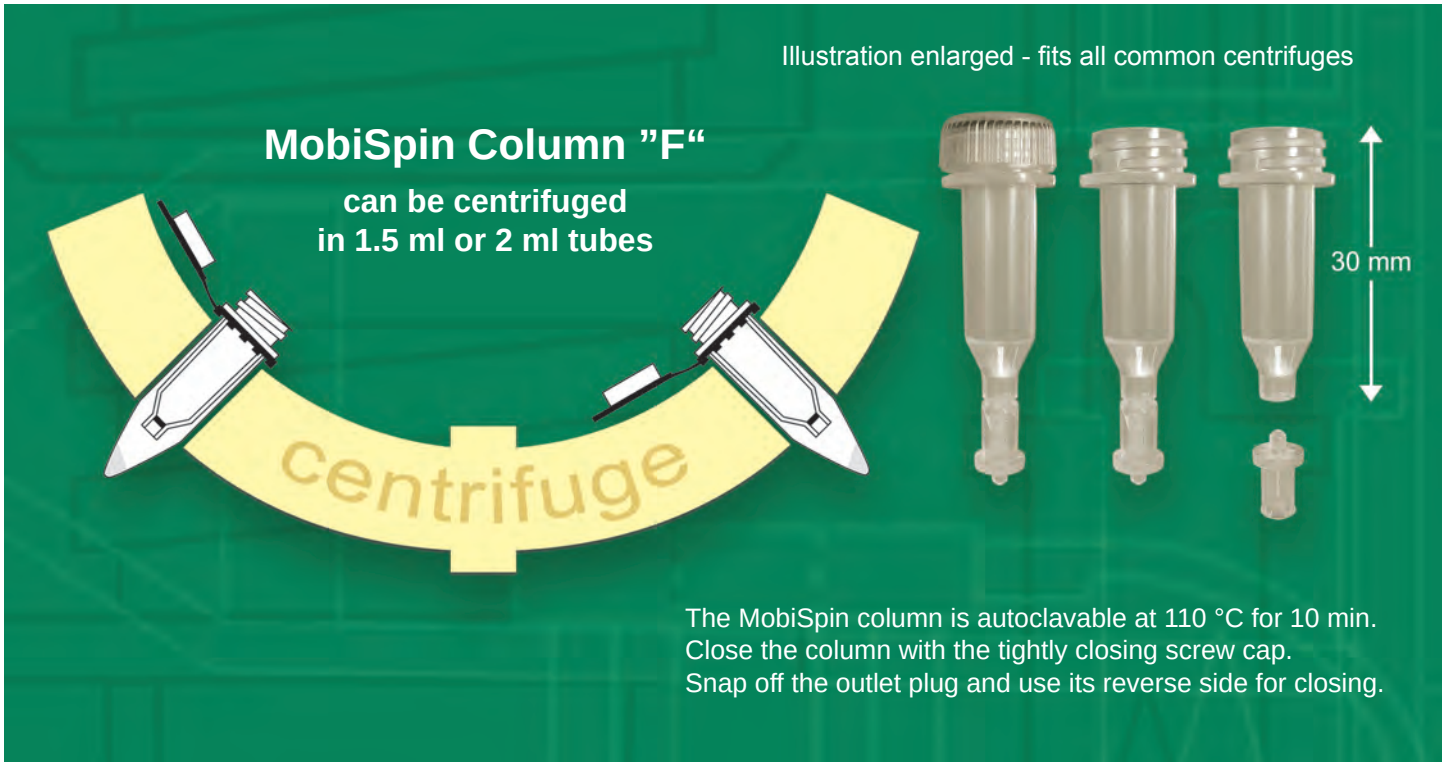
Spin column chromatography offers many advantages over traditional liquid chromatography:

- Comes empty - fill in your own material (matrix, gel slice etc.)
- Easy handling: load sample, spin and collect the purified product
- No sample dilution
- Reproducible results with fast protocols
- One application in less than 4 minutes
- Numerous samples can be processed simultaneously
- Large number of applications
- Compatible with laboratory standard

Background

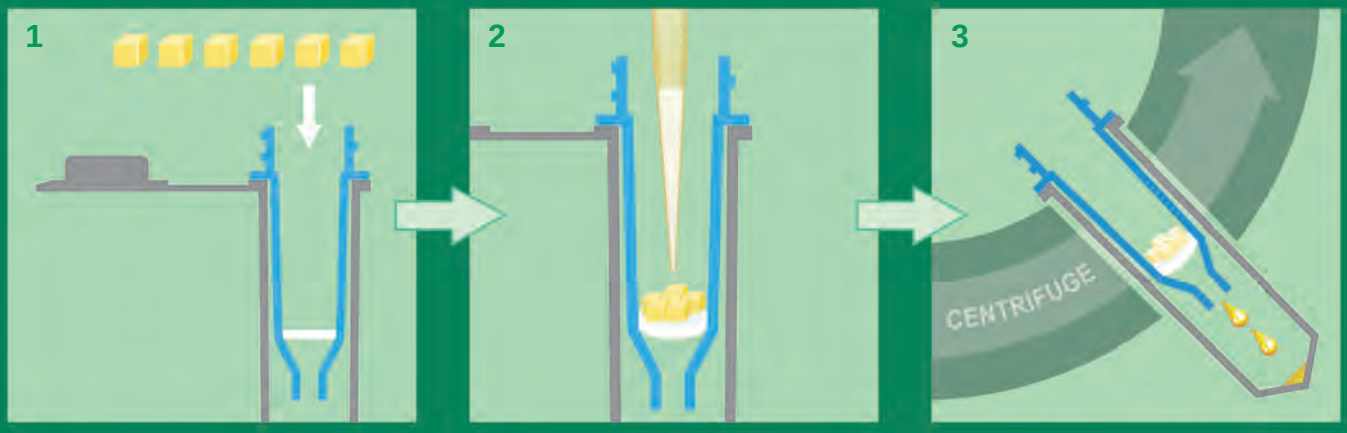
The MobiSpin column ”F“ is designed for a wide variety of applications for nucleic acid and protein extraction and purification. When choosing the MobiSpin column ”F“ for a particular application, a suitable resin must be selected and filled into the mini-column. For an appropriate resin selection, impurity versus sample size and the anticipated yield must be considered.
The MobiSpin column ”F“ comes with inserted small filter of 10 µm pore size, screw cap and snap-off plug. It can be used without matrix (e.g. to extract trypsinated proteins out of a gel slice for Mass Spec analysis) or filled with a matrix of your choice (see also Chapter III).





Special Application

Preparation of trypsinated protein fragments for Mass Spec



For mass spectrometry analysis, proteins are separated by polyacrylamide gel electrophoresis (PAGE) and a single gel slice containing a protein of interest is cut out of the gel. The slice is cut into pieces of approximately 2 x 2 mm and then placed onto the inserted filter of a MobiSpin column ”F“ (M105210S) (1). After several washing and drying steps involving centrifugation, trypsin is added to the gel pieces inside of the column, diffuses into them and digests the protein overnight at 37 °C into small fragments (2). The protein fragments are eluted by a few spinning steps (3). After drying the collected spin elutes can be analyzed by mass spectrometry. The complete protocol is available on our web page (www.mobitec.com). The peptide mass fingerprints provided by this protocol are consistent with results obtained by other common mass spec sample preparations.

This protocol will be suitable for proteases other than trypsin as well.

MobiSpin Column ”F“

Order No.	Description	Amount
M105010S	MobiSpin Column ”F“ with fixed outlet plug, inserted small 10 µm filter and screw cap	50
M105210S	MobiSpin Column ”F“ with fixed outlet plug, inserted large 10 µm filter and screw cap	50

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Chapter III

Matrix-filled MobiSpin Columns for Purification of Nucleic Acids

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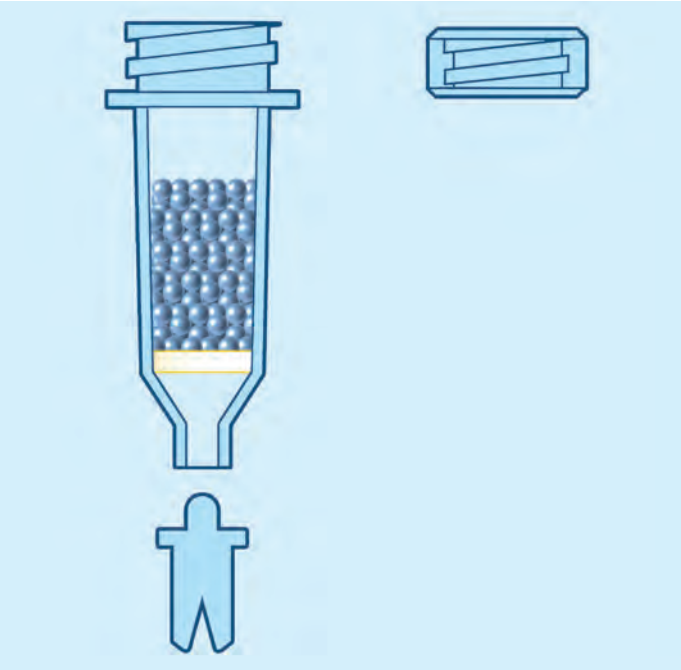
Pre-packed MobiSpin Columns for Separation of DNA and RNA from small Contaminants

The MobiSpin Columns are designed for a wide variety of separation tasks. In this system, a gel matrix consisting of porous particles in aqueous solution is packed into a column and then used for the separation of biomolecules. The filled-in matrix pore size determines which molecules are small enough to enter the matrix beads and which molecules are too large and thus remain outside. Molecules which are larger than the matrix pore size and which cannot enter the matrix beads, appear only in the matrix void volume between the beads. From the inter-bead space they can be recovered by spinning the column in a collection tube in a benchtop centrifuge. Strong spinning elutes the column dead volume without washing and thus without dilution, also in small volumes. Smaller biomolecules located inside of the matrix beads are not eluted by spinning.



Column Usage Guidelines

- 20x rule: For obtaining optimal results, the smallest product being purified should be at least 20 times larger than the largest impurity. A difference in size smaller than 20-fold may affect either purity or yield.
- The larger the pore size of the resin, the greater the purity and the lower the yield of the resulting product. Gel filtration matrices with larger pore size (S-300, S-400) usually retain more product than matrices with smaller pore size (e.g. S-200).
- Non-specific binding: The MobiSpin Columns exhibit only insignificant non-specific binding, allowing purification of samples in the nanogram range.
- Retention: With increasing molecular weight, relative retention decreases.
- For optimizing please use the Sample Volume Guide on page 19.

Application: Removal of small Molecules

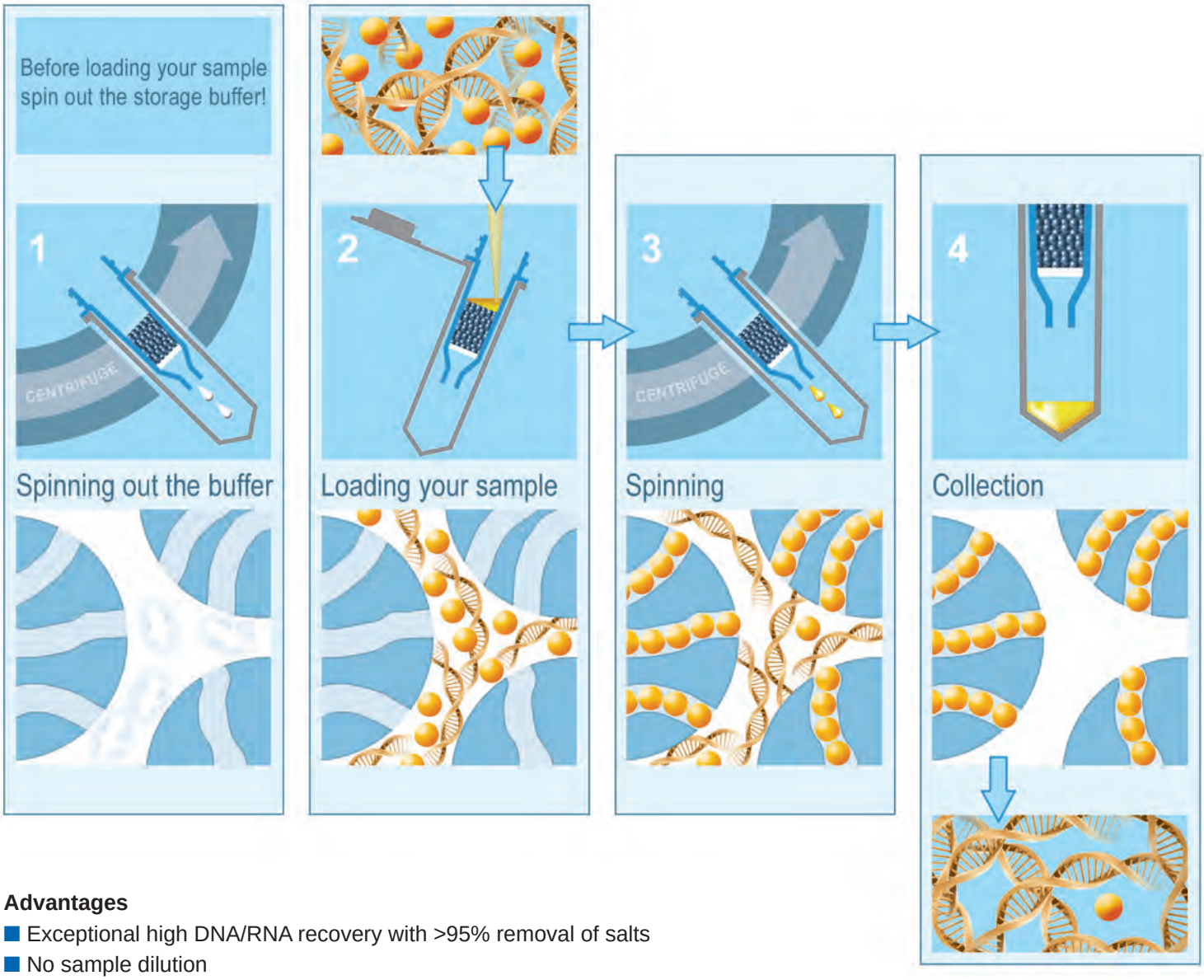
Desalting, removal of ions

Removing salts and other small molecules from biomolecules is often essential for downstream processes. MobiSpin Columns quickly remove small molecules like salt ions from DNA/RNA. The MobiSpin G-50 columns contain a high-performance size-exclusion matrix for effective salt removal and excellent biomolecule recovery. MobiSpin G-50 matrix with a pore size of 700 Da allows hydrated salt ions to enter the matrix beads while DNA/RNA (and most other biomolecules) stay outside.

Removing excess derivatizing agents from modified DNA/RNAs

Removing unreacted dyes from fluorescence labeling reactions

Removing free radiolabel from labeled DNA and RNA



Advantages

- Exceptional high DNA/RNA recovery with >95% removal of salts
- No sample dilution
- No long lasting gravity flow
- No chromatography system
- No column preparation
- Easy handling, multiple sample processing in a few minutes

Applications

MobiSpin Columns for Spin Size Exclusion Chromatography

MobiSpin columns with S-200, S-300 or S-400 matrix can be used to purify plasmid DNA, cDNA, PCR fragments or oligonucleotides.

- DNA purification prior to sequencing
- Oligonucleotide purification after removal of free nucleotides
- Low cost plasmid purification

Oligonucleotides Purification after Synthesis

Oligonucleotides can be purified directly from ammonia deprotection solution using MobiSpin Columns: a synthesized 17-mer oligonucleotide (125 µl) was purified directly using a MobiSpin Column S-200 according to standard protocol. For comparison, a 500 µl aliquot of the same deprotection solution was purified on a Sepahdex G-25 DNA Grade drip column. The quality of DNA obtained with the MobiSpin Column as analyzed by electrophoresis was equivalent to that obtained with the drip column, however purification using the MobiSpin Column took only one fifth of the time required to purify the sample on the drip column.

Removal of ^{32}P-Labeled Nucleotides

MobiSpin Columns can be used to remove large amounts of unincorporated radiolabeled nucleotides. Using the MobiSpin Columns, a 792 bp endlabeled fragment was purified over each of the three MobiSpin Column types. The unincorporated label from 20 µCi reactions was easily removed from the labeled DNA in all cases. In a second experiment, the same 792 bp fragment was used as a template for oligolabeling. Again, the unincorporated labeled nucleotides from 50 µCi reactions were completely and efficiently removed during purification of the reaction products on each type of MobiSpin Column.

Removal of DNA Nucleotides after PCR

Using our MobiSpin Columns, after PCR reactions, DNA nucleotides can be recovered over a wide range of sample loading levels.
In our control experiment, 1 ng of labeled pUC 18 DNA in 50 µl sample volume was purified over each of the MobiSpin Columns S-200, S-300 and S-400. The complete removal of unincorporated, labeled nucleotides was achieved with excellent recovery of labeled DNA.
In a second test, various amounts of labeled DNA (25 - 250 ng) from an endlabeled reaction were combined with large quantities of unlabeled nucleotides (25 - 250 ng). The mixtures were purified over each type of MobiSpin Column. Under these conditions, regardless the resin type, the presence of relatively large amounts of nucleotides had virtually no effect on the yield of longer labeled DNA or on the ability of the column to remove unincorporated nucleotides from the reaction mixture.

Use of MobiSpin S-200 Columns in PCR Template Prep for ssDNA Sequencing

MobiSpin S-200 columns were used in two steps for purifying the PCR template for ssDNA sequencing. Following production of a phosphorylated PCR product, the MobiSpin S-200 column exchanged the buffer and removed the unincorporated nucleotides (1st step). After treating the PCR product with lambda exonuclease (which digests the phosphorylated strand only), a buffer exchange and nucleotide removal were carried out again using a MobiSpin S-200 column (2nd step). After this two-step purification, the purified single-stranded DNA could be sequenced directly.

DNA Purification prior to Sequencing

Plasmid DNA was prepared using a standard alkaline lysis procedure. The miniprep DNA was denatured to the single-stranded form. Then, a volume of 25 µl was purified on each type of MobiSpin Column prior to sequencing. For comparison, CsCl-purified DNA was sequenced in a parallel reaction. Alle three types of MobiSpin Columns yielded results comparable to the CsCl-purified template DNA.

MobiSpin Columns for DNA/RNA purification, separation of larger DNA from mono- and multimers

Sample Volume Guide for S-200, S-300, S-400

Maximum Contaminant Size: 25-mers				
				S-200 (10 µl) S-300 (10 µl) S-400 (25-50 µl)
Maximum Contaminant Size: 18-mers				
			S-200 (10 µl) S-300 (25-50 µl) S-400 (50-100 µl)	S-200 (10 µl) S-300 (25-50 µl) S-400 (50-100 µl)
Maximum Contaminant Size: 8-mers				
	S-200 (25-50 µl) S-300 (25-50 µl) S-400 (50-100 µl)	S-200 (25-50 µl) S-300 (25-75 µl) S-400 (50-100 µl)	S-200 (10-50 µl) S-300 (25-75 µl) S-400 (50-100 µl)	S-200 (10-50 µl) S-300 (25-75 µl)
Maximum Contaminant Size: NTPs/salts				
	S-200 (25-50 µl) S-300 (25-50 µl) S-400 (50-100 µl)	S-200 (25-50 µl) S-300 (25-75 µl) S-400 (50-100 µl)	S-200 (10-50 µl) S-300 (25-75 µl) S-400 (50-100 µl)	S-200 (10-50 µl) S-300 (25-75 µl)
Minimum Product Size:	>20-mers	>50-mers	>200-mers	>500-mers

The sample volumes in the table have been tested for each of the matrices S-200, S-300 and S-400. The recommended sample volume, as well as the matrix suited best for your particular application, can be found at the intersection of the minimum product size (horizontal) and the estimated maximum contaminant size (vertical).
For example, if you want to separate an 8-mer contaminant (3rd row) from your 100 bp molecule (50mer < 100 bp < 200mer; 2nd column), please go to the third row and second column of the table and, dependend on your sample volume, select S-200 (if your sample volume is 25-50 µl), S-300 (if your sample volume is 25-75 µl) or S-400 (if your sample volume is 50-100 µl).

This table is intended to be only a general guideline. The final selection of column type depends on your specific application. If you are uncertain about the optimal volume, apply a sample volume of 50 µl.

Standard Protocol

- Resuspend the resin in the column by vortexing
- Open the bottom plug and loose the cap one fourth turn
- Place the column in a 1.5 ml screw-cap microcentrifuge tube for support.
- Pre-spin the column 1 minute at 800 x g in a microcentrifuge.
- Place the column in a new 1.5 ml tube and slowly apply the sample (10 -100 µl) to the top of the resin, being carefull not to disturb the bed.
- Spin the column 2 minutes at 800 x g. The purified sample is collected in the bottom of the support tube.

MobiSpin Columns are available with three different Sephacryl® resins: S-200, S-300 and S-400. Furthermore, MoBiTec offers MobiSpin Columns filled with Sephadex® G-50 resin. The matrix selection depends on distinct factors, e.g. the sample volume, the size and three-dimensional structure of the product, the g-forces used in the purification process, the resin used as well as the depth of the resin bed.

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Specifications

MobiSpin S-200 columns: (SC0200)	20 columns, pre-packed with Sephacryl® S-200 HR resin and equilibrated in 10 mM Tris/HCl, 1 mM EDTA buffer at pH 7.6
MobiSpin S-300 columns: (SC0300)	20 columns, pre-packed with Sephacryl® S-300 HR resin and equilibrated in 10 mM Tris/HCl, 1 mM EDTA buffer at pH 7.6
MobiSpin S-400 columns: (SC0200)	20 columns, pre-packed with Sephacryl® S-400 HR resin and equilibrated in 10 mM Tris/HCl, 1 mM EDTA buffer at pH 7.6
MobiSpin columns set: (SC0234)	30 pre-packed columns, (10 of each of the above resins) equilibrated in 10 mM Tris/HCl, 1mM EDTA buffer at pH 7.6
MobiSpin G-50 columns: (SC0500)	20 columns, pre-packed with Sephadex® G-50 resin and equilibrated in 10 mM Tris/HCl, 1mM EDTA, 100 mM NaCl buffer at pH 8.0

Equilibrated and ready-to-use

The column caps and their corresponding labels are color-coded for easy identification:

MobiSpin S-200 columns:	MobiSpin S-300 columns:	MobiSpin S-400 columns:	MobiSpin G-50 columns:
Red cap	**Green cap**	**Yellow cap**	**Neutral cap**

Order Information, Shipping and Storage

Order No.	Description		Amount
SCO200	MobiSpin S-200	pre-packed with 400 µl matrix	20 columns
SCO210	MobiSpin S-200	pre-packed with 400 µl matrix	100 columns
SCO300	MobiSpin S-300	pre-packed with 400 µl matrix	20 columns
SCO310	MobiSpin S-300	pre-packed with 400 µl matrix	100 columns
SCO400	MobiSpin S-400	pre-packed with 400 µl matrix	20 columns
SCO410	MobiSpin S-400	pre-packed with 400 µl matrix	100 columns
SCO234	MobiSpin S-200, S-300, S-400	pre-packed with 400 µl matrix	3 x 10 columns
SCO500	MobiSpin G-50	pre-packed with 500 µl matrix	20 columns
SCO510	MobiSpin G-50	pre-packed with 500 µl matrix	100 columns

Shipped at RT; store at 4 °C

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"Quo fata vocant"

Chapter IV

Compact Reaction Columns (CRC)
Mobicols with Enzymatically Active Matrices

CRC with Immobilized Proteases

CRC with Immobilized Nucleases

CRC with other Immobilized Enzymes

Custom Immobilization

Mo Bi Tec®
MOLECULAR BIOTECHNOLOGY

Immobilized Enzymes for solid Phase Enzyme Reactions

Compact Reaction Columns (CRC) are small volume columns (Mobicols) containing a matrix with covalently immobilized enzymes. The patented immobilization chemistry was developed in cooperation with the Max Planck Society. It enables us to offer enzymes bound to F7m and G3m matrices with high enzymatic densities. The high enzyme densities of the CRCs result in fast substrate to product turnover. Enzyme reaction occurs when the substrate is loaded onto the column. The sample is recovered from the column quantitatively either by elution or by centrifugation. Since the enzymes are covalently bound, they remain in the column after reaction and product elution, making the CRC reusable. The eluted product solution is free of enzyme and does not require enzyme removal steps. This results in shorter lab protocols and handling procedures.

Features

- Covalently bound enzymes
- No product contamination
- Suitable for small and large volumes
- Luer-lock connection to reservoir possible
- Short reaction protocol
- CRC is reusable
- Simple product recovery by centrifugation or elution
- Convenient washing enabled/possible
- High enzymatic densities

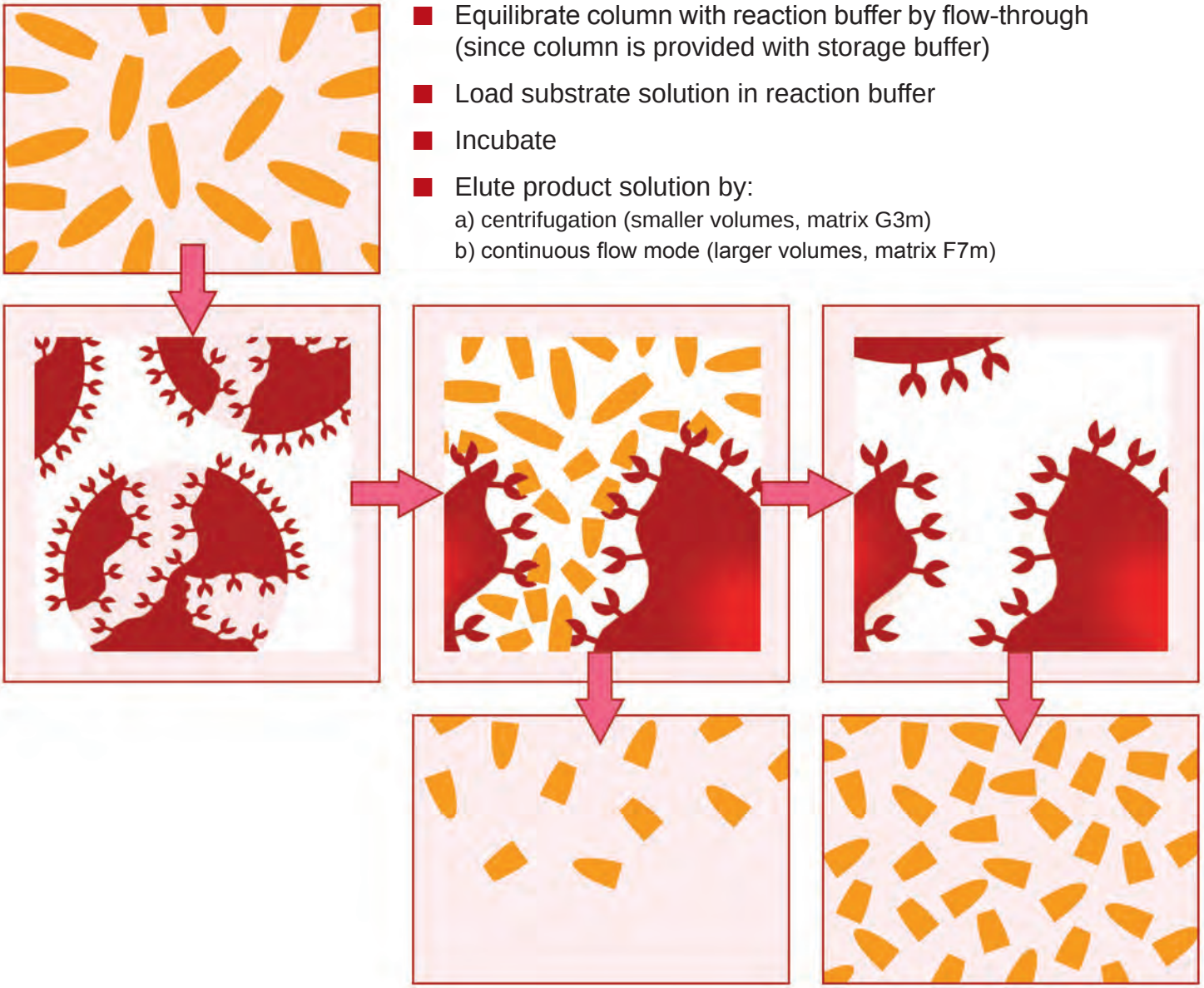
Product Description
The immobilized enzymes are stable in aqueous media at a pH range of 5 to 10 and column bleeding is negligible. The „stiff“ linkers, which keep the enzymes from the matrix surface, effectively eliminate steric hindrance. This results in high activity of enzymes in the immobilized state.
Column elution characteristics vary depending on the nature of the matrix used for immobilization.

- Columns with enzymes immobilized on matrix F7m are designed for applications involving either large substrate quantities, large volumes and/or large molecules.
- Columns with enzymes immobilized on G3m matrix are designed for applications involving small substrate quantities, small volumes and/or small molecules. Samples with a molecular weight ≥10^3 Daltons are recovered in the void volume when loaded on these columns.

This well established technology allows you to expose your reaction solutions to very high concentrations of modifying enzymes. The exposure to high enzyme concentrations allows very short reaction times.

Compact Reaction Columns (CRC)
Immobilized enzymes are supplied in versatile compact reaction columns (CRC) which fit into 1.5 or 2 ml microcentrifuge tubes. The columns have Luer-lock fittings, allowing direct syringe application of substrate solution, continuous flow processing of bulk solutions, or application of pressure for recovering the substrate. For small substrate volumes (approx. 50 µl or less), most enzyme columns can be spun dry in benchtop centrifuges for fast, effective recovery.

Easy handling, brief procedure in 4 steps



- Equilibrate column with reaction buffer by flow-through (since column is provided with storage buffer)
- Load substrate solution in reaction buffer
- Incubate
- Elute product solution by:
  a) centrifugation (smaller volumes, matrix G3m)
  b) continuous flow mode (larger volumes, matrix F7m)

Advantages

- Very high enzyme activities
- No enzyme removal
- G3m: no sample dilution
- No long lasting gravity flow
- No chromatography system
- Easy handling, multiple sample processing in a few minutes

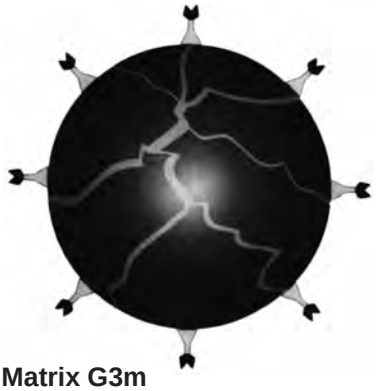
Matrix Characteristics

Two different immobilization matrices are available: F7m and G3m. They have different pore characteristics, as described below.

F7m
Large pores-
high activity!

Matrix F7m

Matrix F7m has large pores. Molecules with up to 10^7 Dalton molecular weight (most enzymes and substrates) can enter these pores. The total surface of the material (including the surface inside the pores) is very large, resulting in an extremely high enzyme activity on the matrix. The product, which also enters the pores, has to be washed out of the matrix, leading to a dilution of the sample solution by additional 200 µl to 500 µl, depending on the substrate. If a dilution of the sample solution by 200 µl or more cannot be tolerated, matrix G3m should be used.



G3m
Small pores-
no sample dilution!

Matrix G3m

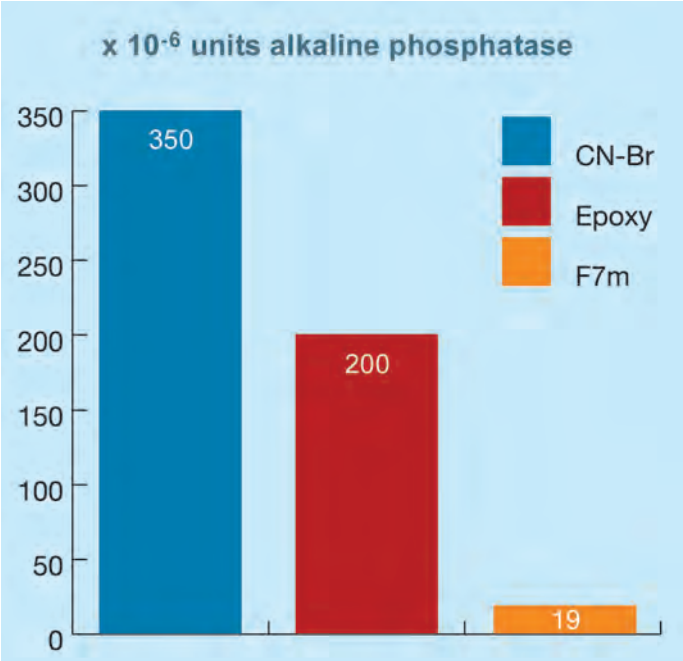
G3m has small pores; this results in excellent recovery characteristics (i.e. complete recovery in very small volumes). Molecules larger than 10^3 Dalton molecular weight (larger peptides, proteins and nucleic acids) cannot enter these pores. The total surface area of the material (only the outside of the spheres) is smaller than for F7m resulting in a smaller enzyme activity on the matrix. The immobilized enzymes as well as most substrates reside only in the volume between the matrix beads (in the dead volume). Thus, the sample solution can be completely removed by centrifugation without dilution. The design of the CR columns makes centrifugation handling easy. Sample solutions down to 20 µl can be treated and recovered.

Immobilization Chemistry

The immobilization chemistry has the particular advantage of being extremely stable in aqueous solution in the pH range 5 to 10. Bleeding-out is negligible. The rather stiff linkers separate the enzymes from the matrix surface thus eliminating steric hindrance. In the immobilized state the enzymes retain a high activity.

Bleeding out in Comparison to Other Immobilization Chemistries

1,000 units alkaline phosphatase were immobilized on the same volume of different activated resins. 200 µl Tris/HCl buffer pH 8.0 were applied on the columns for 20 minutes and recovered. The activity of the alkaline phosphatase in the sample was assayed. The relative amounts are shown in the figure below.



Comparison of three different matrices. According to these results, F7m is the matrix of choice, since bleeding out is significantly reduced compared to CNBr or Epoxy matrices.

Columns, Filters and Buffers

Our 1 ml Mobicol column is especifically designed for this purpose. The enzymes are immobilized on 200 µl matrix which is placed between two filters. The lower filters in the G3m columns have a pore size of 35 µm, in F7m columns the lower filters have 10 µm pore size. All upper filters have 90 µm pore size. The filter material is of polyethylene. The enzyme matrix is delivered in storage buffer. Reaction, washing and storage buffers are provided for the first applications. All buffers are specified on the detailed data sheets provided with the columns.
The amount of liquid, which can be spun out of the CR-column to be replaced by new solution, is 90 µl for F7m matrix and 76 µl for G3m matrix.

Effect of pH

The protein coupling to the matrix is stable in the range of pH 5 to pH 10. For short time periods (up to a few hours) the columns can be used also in the range pH 4 to pH 11. The immobilized enzymes should not, however, be stored below pH 5 or above pH 10. At extreme pH values the enzyme/matrix binding will degenerate.

Biological Activity

DNA plasmids are biologically as active (in transformation) after passage through a CR-column (Proteinase K, RNase A) as the untreated molecules.

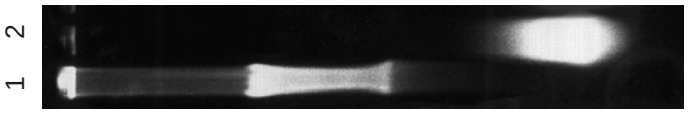
Applications

Removal of genomic DNA

Immobilized DNase I on G3m matrix is a useful tool for the removal of contaminating genomic DNA during RNA purification for single-cell cDNA-PCR: Standard DNase I digestion of RNA prepared from a single cell often leads to loss of material due to the required extraction step. Therefore, a method eliminating phenol extraction is extremely helpful. Using immobilized DNase I instead of soluble enzyme allows the efficient amplification of cDNA by PCR at the single-cell level in the absence of contaminating DNA.

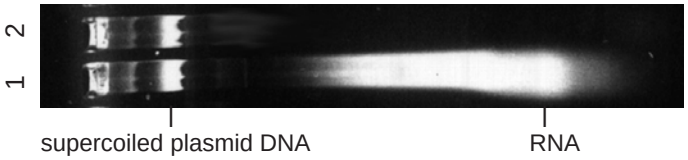
PCR is a process covered by patents owned by Hoffmann La-Roche.

DNA digestion in a column



Immobilized DNase I on matrix G3m Agarose gel showing DNA before (lane 1) and after (lane 2) treatment on a DNase I column. The DNA is completely digested on the column.

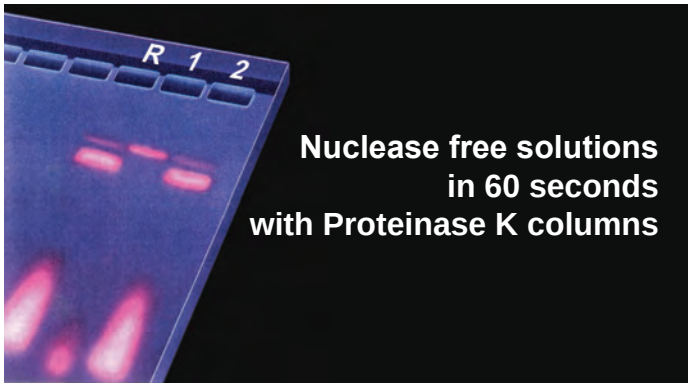
Easy digesting RNA during plasmid preps without phenol extractions



Agarose gel after separation of a clear lysate solution of a plasmid preparation containing DNA (supercoiled and nicked) and RNA (lane 1). Lane 2: Same solution after passage through a RNase G3m column. While the DNA remains unchanged the RNA is digested and cannot be detected.

Linear vector DNA dephosphorylation using an alkaline phosphatase G3m CR-column

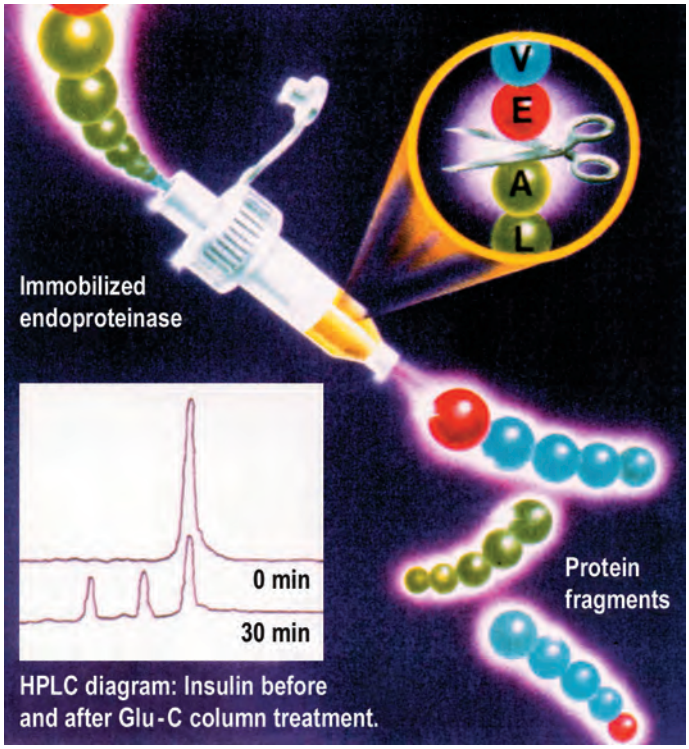
When cloning DNA fragments into plasmids such as expression vectors (i.e. plasmids up to 10 kb), the background resulting from recircularized plasmid vectors has to be minimized. This can be done by an alkaline phosphatase treatment of the linearized plasmid. Both ends will be dephosphorylated, thus preventing recircularization. The DNA fragments to be cloned, generated by restriction enzyme digestion, will provide the 5´terminal phosphate and thus ligate efficiently to the vector. Following plasmid dephosphorylation, the phosphatase activity must be removed before ligation in order to prevent dephosphorylation of the insert. To overcome this problem CR-columns with immobilized alkaline phosphatase provide dephosphorylated DNA ready for ligation without phosphatase activity contamination.



A DNA and RNA solution (reference, lane R) is contaminated with nucleases. After passage through a Proteinase K CR column, both solutions are incubated at 37°C for one hour, followed by agarose gel analysis. Untreated solution is digested (lane 1) while the recovered sample from the column (lane 2) is free of degradation.

Insulin Box digestion by an endoproteinase Glu-C Compact Reaction Column (matrix G3m)

Since the endoproteinase is immobilized, it is completely retained in the column. Thus, the cleaved protein is entirely free of proteinases (and protease fragments); there is no need for further purification of the product.



Schematic drawing of proteins cleaved within a compact reaction column containing endoproteinase Glu-C immobilized on matrix G3m.
Small insert: 30 µg of insulin Box were loaded on an endoproteinase Glu-C compact reaction column with matrix G3m. The substrate solution was left in the column for 30 minutes and was analyzed by reverse phase HPLC. Peptides were detected at wavelength 215 nm.

Order Information, Shipping & Storage

Order No.	Description	Amount
Immobilized Proteinases (in CRC)		
P5101	Endoproteinase Glu-C (Prot.V8) (one column with 200 µl matrix F7m)	900 U
P3102	Endoproteinase Glu-C (Prot.V8) (one column with 200µl matrix G3m)	22 U
P5401	Papain (one column with 200 µl matrix F7m)	23 U
P3402	Papain (one column with 200 µl matrix G3m)	0.6 U
P5121	Pepsin (one column with 200 µl matrix F7m)	16 mAnson U
P3122	Pepsin (one column with 200 µl matrix G3m)	0.4 mAnson U
P5501	Proteinase K (one column with 200 µl matrix F7m)	27 mAnson U
P3502	Proteinase K (one column with 200 µl matrix G3m)	0.7 mAnson U
P5301	TLCK-α -Chymotrypsin (one column with 200 µl matrix F7m)	55 U
P3302	TLCK-α -Chymotrypsin (one column with 200 µl matrix G3m)	1.4 U
P5701	TPCK-Trypsin (one column with 200 µl matrix F7m)	10,200 St-U
P3702	TPCK-Trypsin (one column with 200 µl matrix G3m)	260 St-U
Immobilized Nucleases in CRC (except N3403)		
N5401	DNase I (one column with 200 µl matrix F7m)	3,500 U
N3402	DNase I (one column with 200 µl matrix G3m)	88 U
N3403	DNase I (kit with 200 µl matrix and 5 empty columns)	88 U
N5101	RNase A (one column with 200 µl matrix F7m)	50 Kunitz-U
N3102	RNase A (one column with 200 µl matrix G3m)	2.5 Kunitz-U
Immobilized Other Enzymes (in CRC)		
A5201	Alkaline phosphatase (CIP) (one column with 200 µl matrix F7m)	1,000 U
A3202	Alkaline phosphatase (CIP) (one column with 200 µl matrix G3m)	100 U
A5101	β-Galactosidase (one column with 200 µl matrix F7m)	600 U
A3102	β-Galactosidase (one column with 200 µl matrix G3m)	15 U

shipped at RT; store at 4°C

Note: Never freeze a CR-column!

A detailed handbook including all protocols is provided with the product. It is also available for download on our website www.mobitec.com.

All Columns are delivered with

- 200 µl immobilized enzyme matrix
- concentrated buffers for the first applications
- our CRC handbook about immobilized enzymes
- a data sheet with the description of the immobilized enzyme, the buffer composition and the protocol for the use of the column.

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Mobicols

SEPARATION

ISOLATION

TREATMENT

PURIFICATION

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