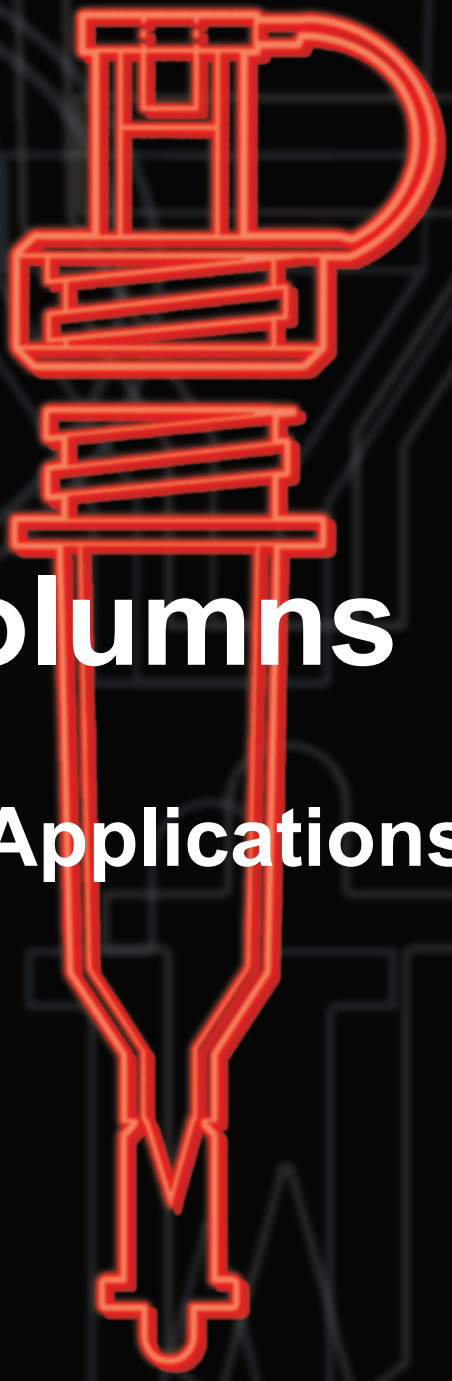


Mobicols - Columns

Tools for diverse Lab Applications

SEPARATION

ISOLATION

TREATMENT

PURIFICATION

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Introduction

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**).

Mobicols filled with a Protein A or Protein G matrix are useful for the purification of antibodies from cell culture supernatant, plasma or serum. Prefilled Mobicols are available in Starter Kits with all required buffers, columns and accessories (**Chapter IV**).

Applying Mini Columns, you can take advantage of highly active immobilized enzymes, packed into small Compact Reaction Columns (CRC) (**Chapter V**).

Chapter I

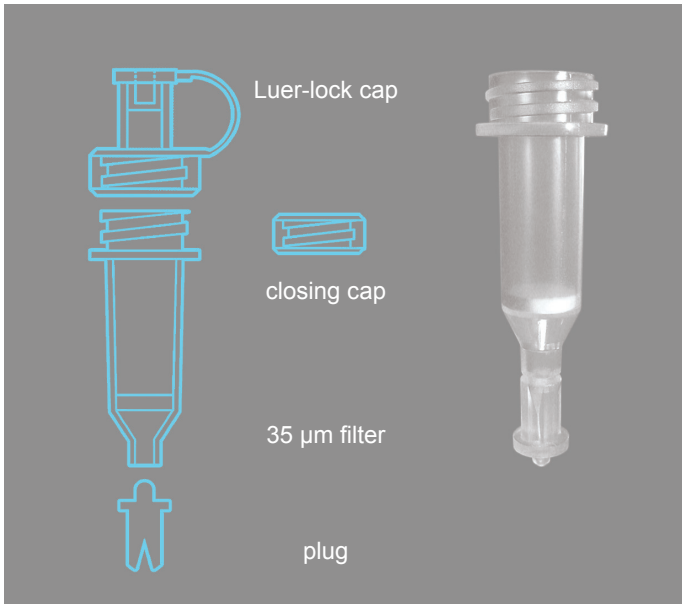
Mobicol – A Practical Tool for every Lab

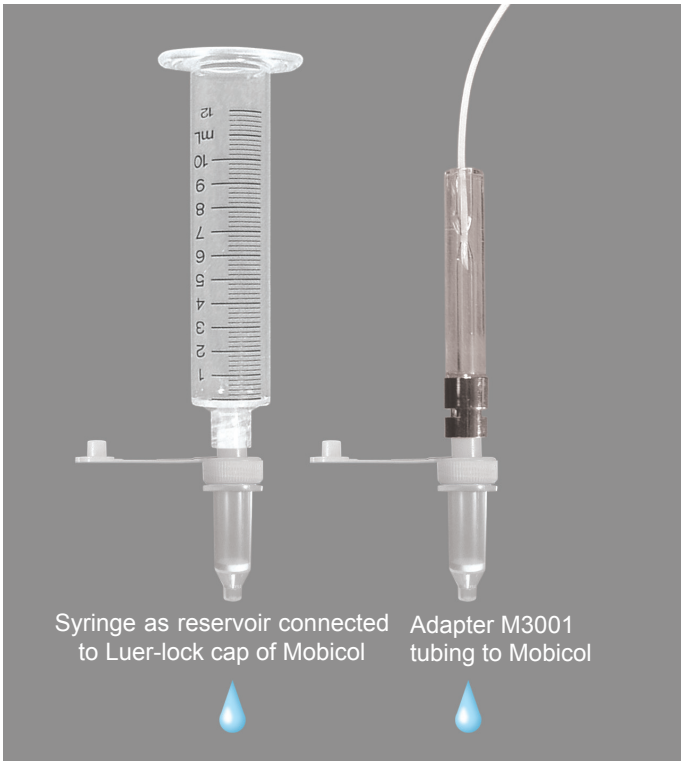
Mo Bi Tec®
MOLECULAR BIOTECHNOLOGY

Mobicol “F”

Mobicols are plastic columns with particular handling advantages:

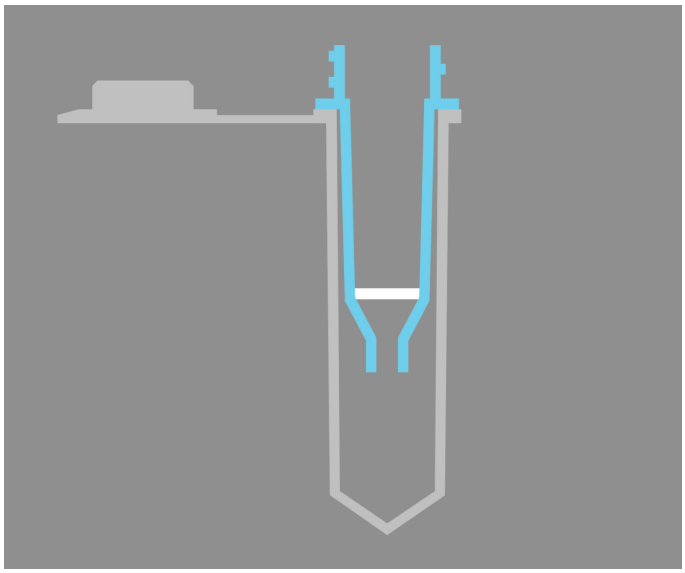
- Mobicols are compatible with laboratory standards
- Mobicols can be centrifuged in microcentri-fuges
- Mobicols have a Luer-lock adapter
- Smallest und largest volumes can be treated easily



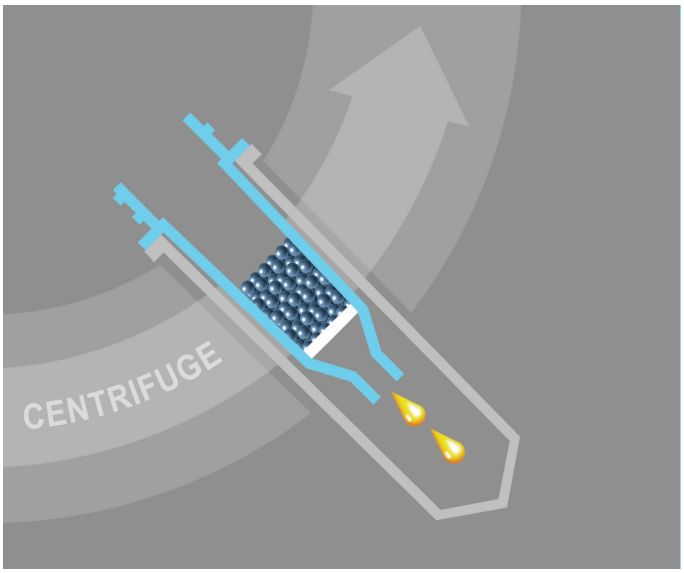


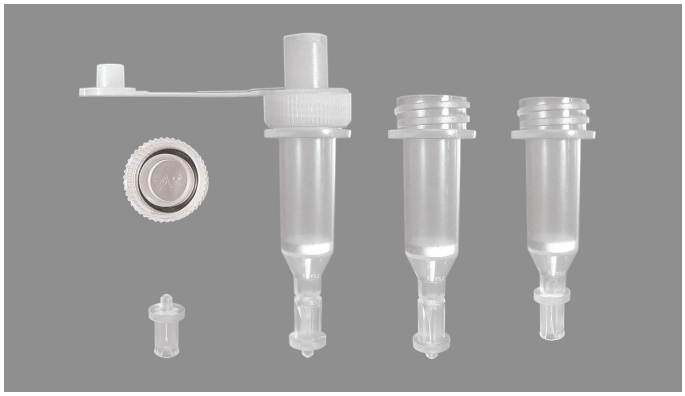
Filter of 35 µm pore size is inserted. A separately available upper filter (Order No. M523515) conveni-ently caps the resin bed.
Mobicol “F” can be closed tightly with a screw-on cap and a snap-off plug at the outlet.
Mobicol “F” can be autoclaved at 110 °C for 10 minutes.

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



Mobicol “F” can also be used as Spin Column





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.

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**

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
- Empty columns for other filter pore sizes available

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 filters at 110 °C) for 10 min

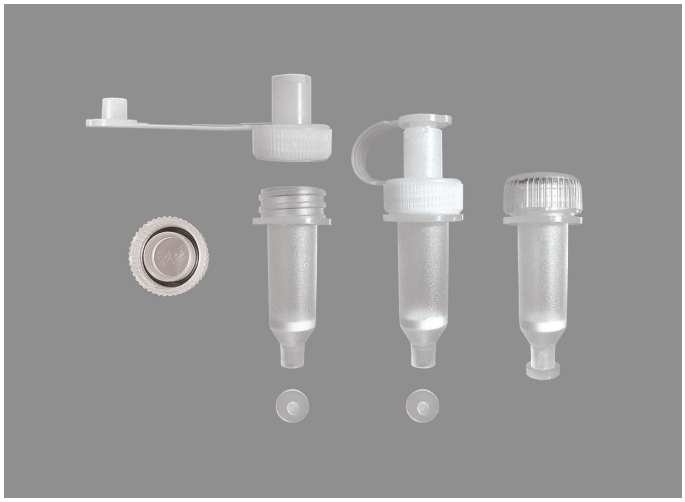
Order Information

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
M2290	Filter (large) 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, or β-galactosidase **(Chapter V)**.

Mobicol “Classic”

Use for Affinity Chromatography (M1002) with inserted filters (large)



Use as Spin Column (M1003) with inserted filters (small)

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

Ask for Free Samples!

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

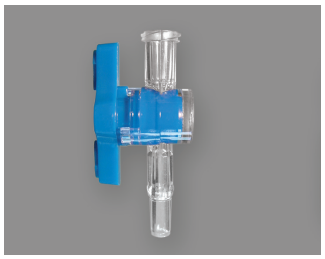
The polypropylene Luer adapter (M) (M3003) for tubing connection to the inlet of the column allows a flow-trough mode at low pressure, whereas the Luer adapter (F) (M3004) is suitable for a tubing connection to the outlet of the column. Furthermore, the Luer adapter (M/M) (M3007) facilitates the connection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

M3001 Tubing-to-Luer-lock adapter (connected to Mobicol "Classic" (M1002) with inserted large filter; not enclosed)

M3003
Luer adapter (M) for Luer-lock cap

M3004
Luer adapter (F) for outlet



M3006
Stopcock, one-way, Luer-lock (F)

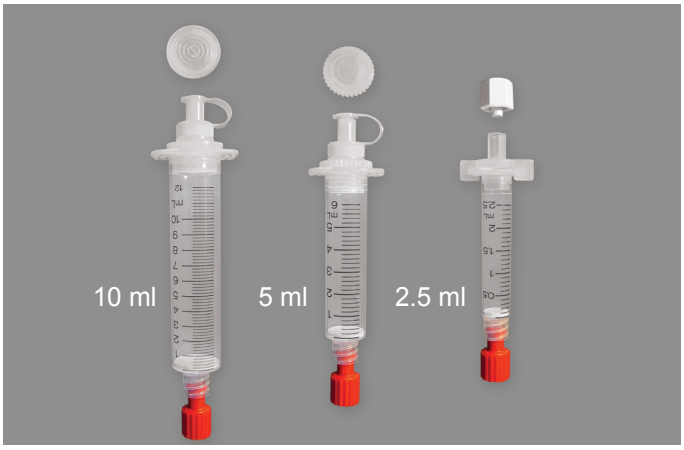
M3007
Luer-lock adapter (M/M)

Column Accessories

Order No.	Description	Amount
M3001	Tubing-to-Luer-lock adapter (material outside: metal; inside: glass and PTFE; with 1 m PTFE 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 Mobicol M1002	50
M3006	Stopcocks, one-way, Luer-lock (F)	4
M3009	Luer-lock caps	50

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

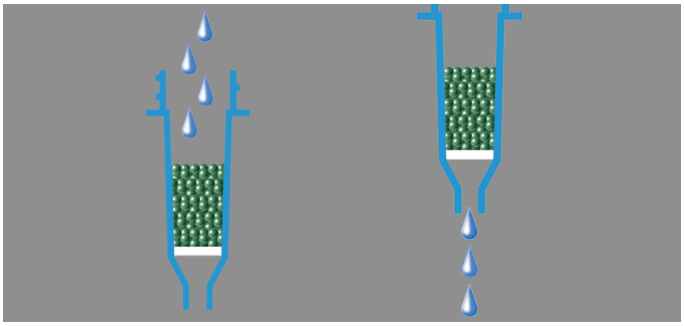
Column sets

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

Related Products: Matrices

A wide range of matrices, e.g. for the purification of His- or GST-tagged proteins, antibodies, nucleotide-binding proteins and phosphor-aminoacid-binding proteins, are available to be used in our Mobicols or plastic columns.

An overview is listed in the table below. Please contact us for more information or visit our website.



Order No.	Description	Amount
AC-201-10-JB	6% CL-Glutathione ChroMatrix™	10 ml
AC-303-25-JB	High Density Nickel Agarose	25 ml
AC-304-25-JB	Low Density Nickel Agarose	25 ml
AC-305-25-JB	High Density Zinc Agarose	25 ml
AC-202-10-JB	6% CL-Nickel ChroMatrix™	10 ml
AC-309-5-JB	Protein A Agarose	5 ml
AC-101S-JB	Immobilized gamma-Aminophenyl-ATP (C10-spacer), Agarose	1 ml
AC-102S-JB	Immobilized gamma-Aminophenyl-ATP (no spacer), Agarose	1 ml
AC-106S-JB	Immobilized gamma-Amino-octyl-GTP, Agarose	1 ml
AC-107S-JB	Immobilized gamma-Amino-octyl-UTP, Agarose	1 ml
AC-108S-JB	Immobilized gamma-Amino-octyl-CTP, Agarose	1 ml
AC-109S-JB	Immobilized gamma-Amino-octyl-ITP, Agarose	1 ml
AC-110S-JB	Immobilized gamma-Amino-octyl-XTP, Agarose	1 ml
AC-111S-JB	Immobilized gamma-Amino-octyl-dATP, Agarose	1 ml
AC-115S-JB	Immobilized gamma-Amino-octyl-dTTP, Agarose	1 ml
AC-119S-JB	Immobilized gamma-Amino-hexyl-CTP, Agarose	1 ml
AC-120S-JB	Immobilized gamma-Amino-hexyl-ITP, Agarose	1 ml
AC-121S-JB	Immobilized gamma-Amino-hexyl-XTP, Agarose	1 ml
AC-122S-JB	Immobilized gamma-Amino-hexyl-dATP, Agarose	1 ml
AC-124S-JB	Immobilized gamma-Amino-hexyl-dUTP, Agarose	1 ml
AC-137S-JB	Immobilized O-Phospho-L-Serine (C10-spacer), Agarose	1 ml
AC-139S-JB	Immobilized O-Phospho-L-Threonine (C10-spacer), Agarose	1 ml
AC-103S-JB	Immobilized O-Phospho-L-Tyrosine (C10-spacer), Agarose	1 ml
AC-104S-JB	Immobilized O-Phospho-L-Tyrosine (no spacer), Agarose	1 ml
AC-142S-JB	Immobilized 2'/3'-EDA-m7GTP, Agarose	1 ml
AC-144S-JB	Immobilized 2'/3'-EDA-ADP, Agarose	1 ml
AC-145S-JB	Immobilized N6-(6-Amino)hexyl-AMP, Agarose	1 ml
AC-146S-JB	Immobilized 8-Amino-hexyl-cAMP, Agarose	1 ml
AC-147S-JB	Immobilized 2'-EDA-cAMP, Agarose	1 ml
AC-148S-JB	Immobilized 2'-EDA-cGMP, Agarose	1 ml

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Chapter II

Spin Columns for your own Matrices or other Compounds

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

MobiSpin Column “F”

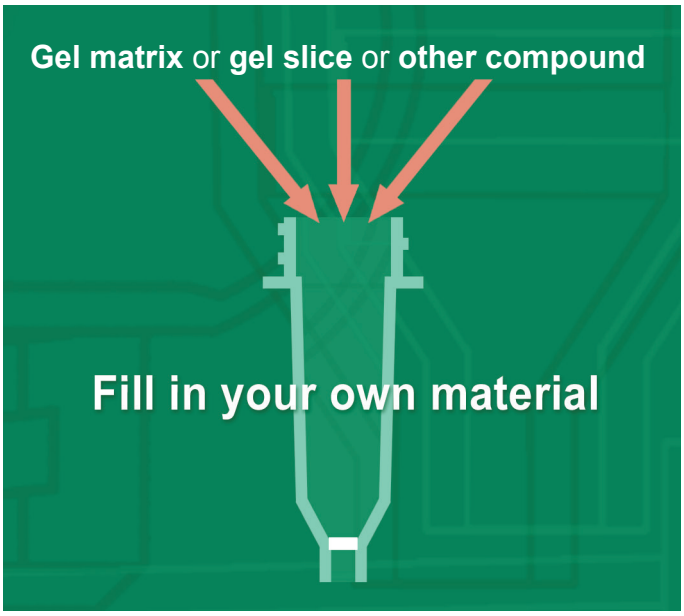
Advantages

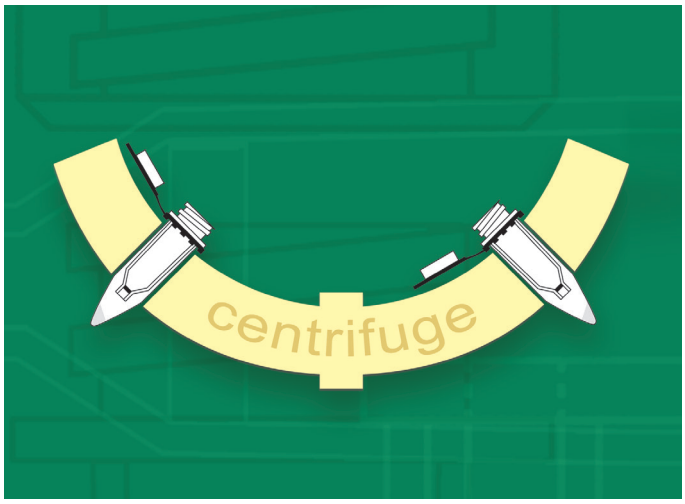
Spin column chromatography offers many advantages over traditional liquid chromatography:

- 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

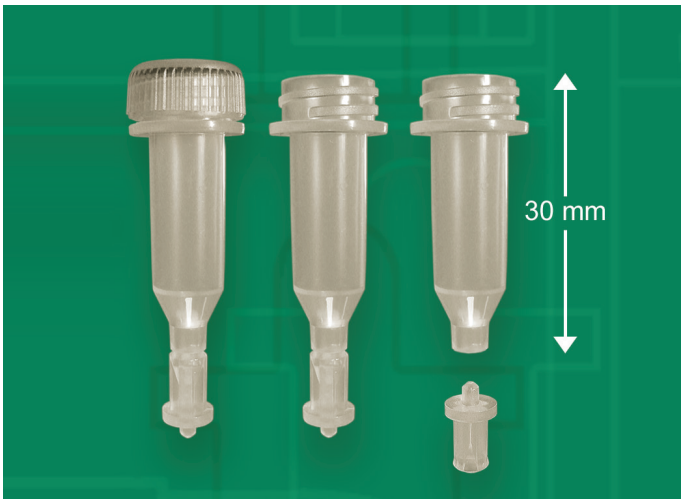
Background

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 or large 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).





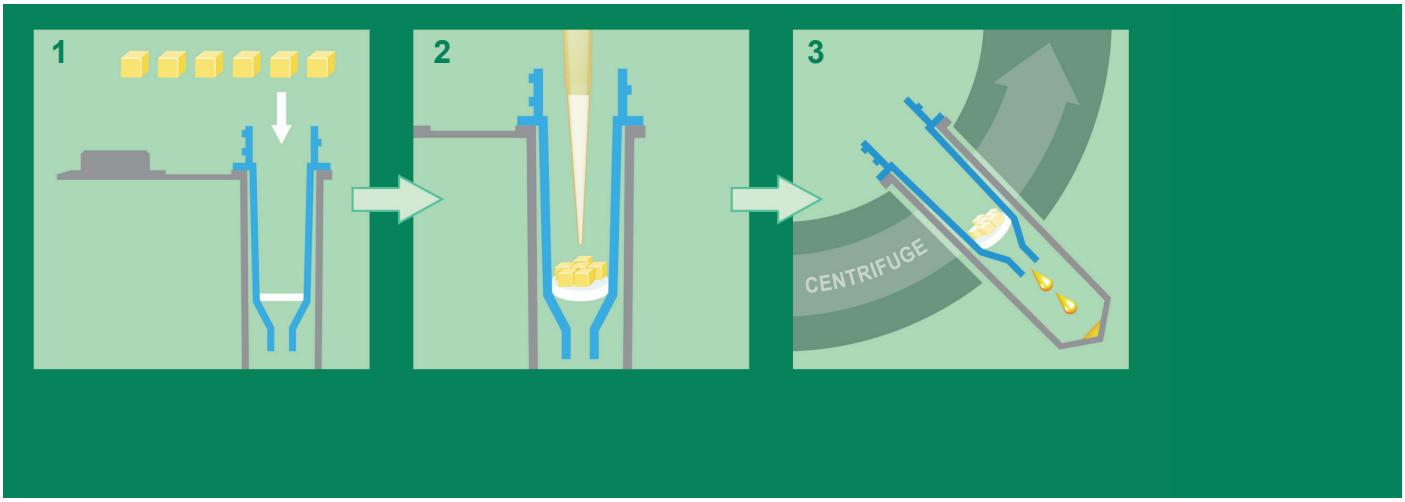
MobiSpin Column “F” can be centrifuged in 1.5 ml or 2 ml tubes



The MobiSpin column is autoclavable at 110 °C for 10 min. Close the column with the tightly closing screw cap.
Snap off the outlet plug and use its reverse side for closing.

Special Application of MobiSpin Column “F”

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.

Ask for Free Samples!

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

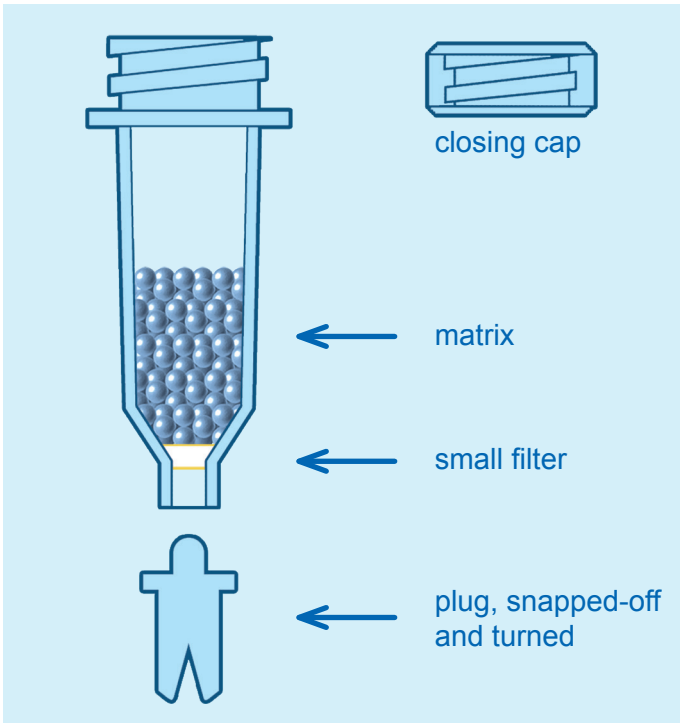
Mo Bi Tec®
MOLECULAR BIOTECHNOLOGY

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

Removing salts and other small molecules from biomolecules is often essential for downstream processes. MobiSpin Columns are developed for rapid and efficient routine separation tasks regarding DNA and RNA.

Features

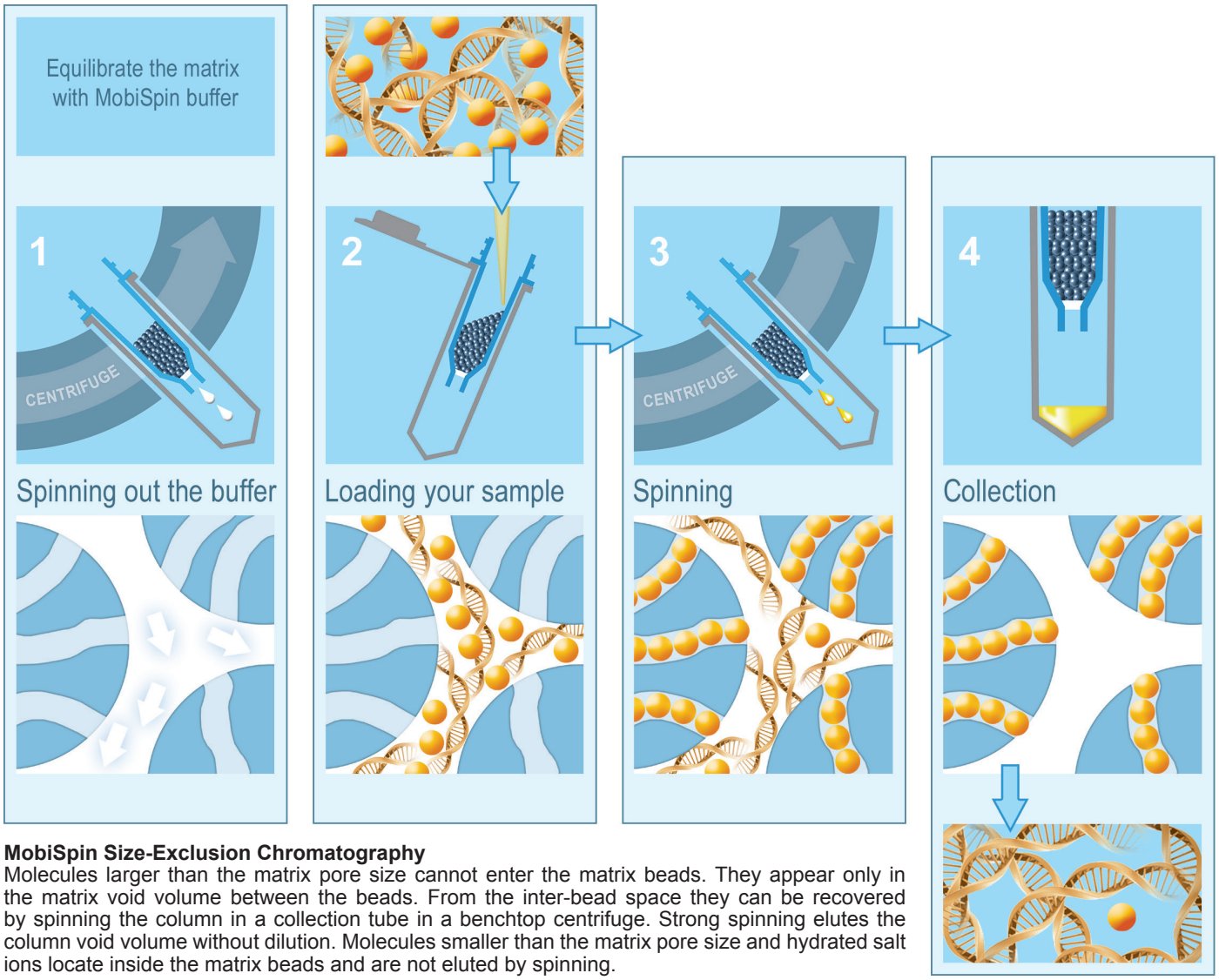
- Compatibility with **laboratory standard**
- Columns are **pre-packed** and **ready to use**
- **Very fast** procedure: Sample loading and recovery in less than 3 minutes
- **Easy handling:**
  1. Equilibrate with MobiSpin or Storage Buffer, spin, and discard the flow through
  2. Load sample, spin, and collect the purified product
- Numerous samples can be **processed simultaneously**
- **Excellent results:** Exceptional high DNA/RNA recovery with > 95% desalting efficiency
- **No sample dilution**



Pre-packed MobiSpin Column
The pre-packed MobiSpin Column is closed with a screw cap and contains a matrix comprised of microscopic beads of a porous material.
The tip of the MobiSpin Column is a fixed snap-off plug. Prior to the first centrifugation step it has to be bent down and removed. The plug can be reused for closing by turning it upside down.

Functional Principle: Size-Exclusion Chromatography

MobiSpin Columns, pre-packed with diverse matrices, are designed for a wide variety of separation tasks including nucleic acid purification. They function by the proven principle of size-exclusion chromatography. The columns combine the effectiveness of gel filtration with speed of centrifugation.
The pore size of the filled-in matrix determines which molecules are small enough to enter the pores of the matrix beads and which molecules are too large. In this way nucleic acid molecules larger than the pore size are excluded from the resin and located in the void volume. These molecules move quickly through the matrix bed when the column is centrifuged. Molecules smaller than the pore size, like hydrated salt ions, enter the pores of the matrix beads and are held back.
A detailed overview on the functional principle is given in the figure below.



MobiSpin Size-Exclusion Chromatography
Molecules larger than the matrix pore size cannot enter the matrix beads. They 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 void volume without dilution. Molecules smaller than the matrix pore size and hydrated salt ions locate inside the matrix beads and are not eluted by spinning.

Applications

Pre-packed MobiSpin Columns for nucleic acid purification are available with three different matrices.

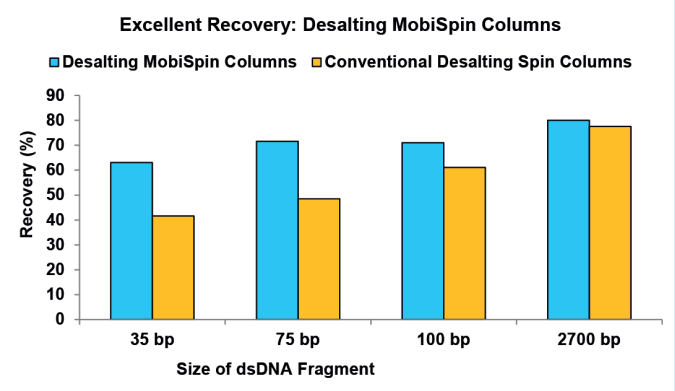
- Silica: Desalting MobiSpin Columns
- Sephacryl®: MobiSpin S-Columns
- Sephadex®: MobiSpin G-Columns

The resins have differing suitability regarding their usage. We recommend these pre-packed MobiSpin columns as rapid and efficient tool for the following applications.

Desalting and Buffer Exchange

Desalting MobiSpin Columns quickly remove small molecules like salt ions from DNA/RNA (even from dsDNA fragments <25 bp and from dNTPs) with good recovery efficiency. The columns are pre-packed with a novel silica resin comprised of uniform microscopic beads of a porous, form-stable material. Since this new matrix material works without moisture expansion, resins are allowed to fall dry without any loss in purification efficiency. This is in contrast to Sephacryl®, Sephadex®, and Sepharose® resins which swell in buffer. Therefore, Desalting MobiSpin Columns give reproducible results of high quality and are much easier to handle. Furthermore, the columns are long-term storable when kept dry.

As an alternative, **MobiSpin G-Columns** pre-packed with conventional Sephadex® G-50 matrix may be used. This hydrophilic matrix swells in buffer and acquires its chromatographic properties only under wet conditions. In this state beads have a pore size of 700 Da that allow hydrated salt ions to enter into the pores, while DNA/RNA > 20 bases and most other biomolecules stay outside.



DNA Recovery of Desalting MobiSpin Columns and Conventional Desalting Spin Columns
In all cases, a high desalting efficiency of > 90% could be achieved. The recovery of large-sized fragments > 2kb was nearly comparable (about 80%) with both kinds of spin columns. But with small-sized fragments ≤ 100 bp the Desalting MobiSpin Columns showed a considerably improved recovery.

Fast Removal of Dye Terminators or Unincorporated Labeled Nucleotides

MobiSpin S-Columns pre-packed with Sephacryl® matrices (S-200, S-300, S-400) or **MobiSpin G-Columns** pre-packed with Sephadex® G-50 matrix are particularly suited for the removal of fluorescent dye dideoxyterminators (e.g., Cy5/Cy3 nucleotides) from cycle sequencing reactions.

Furthermore these columns are convenient for the extraction of unincorporated labeled nucleotides (dye-labeled or radiolabeled dNTPs or ddNTPs) from DNA labeling reactions, e.g., PCR probe labeling, Nick Translation, or DNA end-labeling. The purified DNA is applicable to downstream applications like FISH (fluorescence *in situ* hybridization) or Southern/Northern blotting. The removal of unincorporated labeled nucleotides is a precondition for determining the DNA labeling rate. For good recovery rates, labeled DNA fragments must be at least 20 bp in length.

Pre-packed and ready-to-use

Applications

Removal of dNTPs, Oligos, and Salt

MobiSpin S-Columns pre-packed with Sephacryl® matrices (S-200, S-300, S-400) are particularly suited for purification of PCR and other enzymatic DNA reactions. With these matrices nucleotides, oligonucleotides, and buffer (but not enzymes) will be removed. The larger the pore size of the resin (S 400 > S-300 > S-200), the greater the purity and lower the yield of the product.
For obtaining optimal results please consider: the smallest product being purified should be at least 20 times larger than the largest impurity.

Note:

For choosing the appropriate Sephacryl® matrix, the exact size of the nucleic acid fragment to be purified and the sample volume has to be considered. As a guideline we recommend using MoBiTecs “Sample Volume Guide” that is given in the **MobiSpin S-Columns Handbook** which is available on our website.

Pre-packed MobiSpin Columns - Overview

Column	Matrices	Applications
Desalting MobiSpin	Silica	Desalting and buffer exchange especially of small sized nucleic fragments and dNTPs
MobiSpin S (S-200; S-300; S-400)	Sephacryl®	Buffer exchange, nucleotide removal, plasmid, oligo and PCR reaction purification, removal of unincorporated dyes or dye terminators
MobiSpin G (G-50)	Sephadex®	Removal of unincorporated dyes or dye terminators, desalting and buffer exchange

Sample loading and recovery in less than 3 minutes

Order Information, Shipping and Storage

Silica-Based Columns

Order No.	Description	Amount
SCO100	Desalting MobiSpin	20 Columns
SCO110	Desalting MobiSpin	100 Columns

Including MobiSpin Buffer (10 mM Tris/HCl pH 7.6; 1 mM EDTA) for equilibration; shipped at RT; store columns at RT and buffer at 4 °C

Sephacryl®-Based Columns

Order No.	Description	Amount
SCO200	MobiSpin S-200	20 Columns
SCO210	MobiSpin S-200	100 Columns
SCO234	MobiSpin S-200, S-300, S-400	3x 10 Columns
SCO300	MobiSpin S-300	20 Columns
SCO310	MobiSpin S-300	100 Columns
SCO400	MobiSpin S-400	20 Columns
SCO410	MobiSpin S-400	100 Columns

Equilibrated in MobiSpin Buffer (10 mM Tris/HCl pH 7.6; 1 mM EDTA); shipped at RT; store columns at 4 °C

Sephadex®-Based Columns

Order No.	Description	Amount
SCO500	MobiSpin G-50	20 Columns
SCO510	MobiSpin G-50	100 Columns

Equilibrated in MobiSpin Buffer (10 mM Tris/HCl pH 7.6; 1 mM EDTA) shipped at RT; store columns at 4 °C

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Chapter IV

Mobicols for Affinity Purification of Biomolecules

Fractionation and Purification of Antibodies

Purification of antibodies using immobilized Protein A / Protein G

Immobilized Metal Ion Affinity Chromatography (IMAC)

Purification of recombinant polyhistidine-tagged proteins
using pre-packed Ni-IDA columns

Mo Bi Tec®
MOLECULAR BIOTECHNOLOGY

Protein A and G columns

Advantages

- Time saving: antibody purification in less than 20 minutes
- High-quality controlled glass porous matrix is more rigid and durable than conventional matrices
- Starter kits with required buffers, columns and accessories available
- The Protein A and G columns are ideally suited for the efficient and rapid purification of antibodies with minimal pre-treatment
- Protein A and G columns have good flow properties
- Capacity of these Protein A and G columns for murine IgG at physiological pH and salt concentration are greater than that for other Protein A and G adsorbents

Background

The protein is immobilized on controlled pore glass which is more rigid and durable than conventional polymeric (for example agarose) matrices. This glass also has a very uniform internal pore size distribution which falls within a very narrow range, resulting in uniform rates of diffusion and hence rapid transfer between solution and solid phase. The protein molecules are orientated and immobilized on the glass surface such that pairs of Protein A and G molecules can interact with the binding regions of each of the two Fc portions of the immunoglobulin at the same time. This results in very high binding capacities, especially for those immunoglobulins which typically do not bind well to conventional immobilized Protein A and G and where this bivalent binding is an essential prerequisite (for example mouse IgG_1). The increased capacity does not, however, result in a higher association constant (Ka) and therefore, elution conditions remain unaltered.

Columns with immobilized Protein A and G may be used for many applications including:

- Purification of monoclonal antibodies
- Selective purification of antibody fragments
- Purification or removal of polyclonal IgG from serum
- Separation of IgG sub-classes using a stepwise pH gradient

The plastic columns with immobilized Protein A and G facilitate highly efficient, cost effective purification for both monoclonal and polyclonal antibodies. These columns bind antibodies under binding buffer conditions, the antibodies are eluted from the column under elution buffer conditions, and the columns are then washed and stored under storage buffer. They can be stored and reused many times.

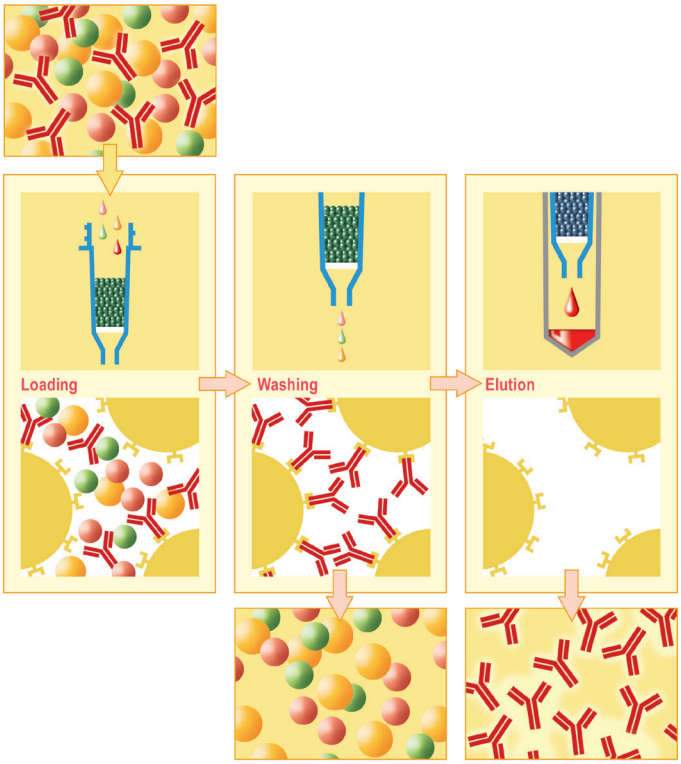
The immobilization of Protein A and G has been developed to satisfy four critical factors:

- Orientation of the ligand
- Distribution of the ligand
- Stability of the immobilized ligand
- Elimination of non-specific surface interactions

Immobilized onto a solid glass support and packed into columns, Protein A and G purifies total IgG from crude protein mixtures such as serum or ascites fluid.

Antibody purification by Protein A and G columns

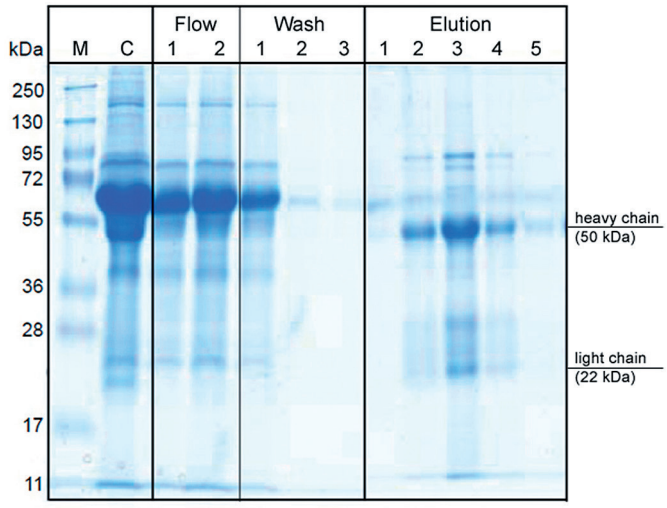
Polyclonal antibodies in plasma or serum, or monoclonal antibodies in cell culture supernatant are loaded onto the Protein A or G columns. There, the Fc domains of the antibodies are captured by the matrix immobilized Protein A or G (Loading) while other proteins are not bound. All contaminants other than the antibodies are washed off (Washing) removing unbound or weakly bound material from the column. Then, the buffer conditions are changed to lower pH values (down to about pH 3) so that Protein A or G cannot bind the Fc antibody domains anymore and the purified antibodies are eluted (Elution).



Example

Purification of polyclonal antibodies from rabbit serum

The elution yield is determined by SDS-PAGE gel analysis of the washing and elution steps



SDS-PAGE analysis of washing and elution steps. M: protein markers, C: loading, Flow: flow through 1 and 2, Wash: washing steps 1, 2 and 3, Elution: elution steps 1, 2, 3, 4 and 5. 1:4 diluted rabbit serum was loaded onto the column (C) and the flow through analyzed (Flow). For washing (Wash), about 5 ml PBS buffer (10 column volumes) was loaded onto the column and the flow through was collected in 1.5 ml aliquots; 3 aliquots were loaded on the gel. For elution (Elution), 5 ml elution buffer was loaded onto the column and the flow through was collected in 0.5 ml aliquots; the first 5 aliquots were loaded on the gel. The large amount of the dominant 65 kDa protein runs through the column and does not bind. The antibody heavy chain (50 kDa) does bind to the column (band present in lane C but not in Flow and Wash lanes). The antibody heavy (50 kDa) and light (22 kDa) chains elute in elution aliquots 2 to 4 and mainly in elution step 3.

Applications

Protein A, Protein G
Protein A and Protein G are bacterial proteins with high specific binding to the Fc (non-antigen binding) domain of many classes of antibodies. Protein A and G can be used to affinity purify immunoglobulins (primarily IgGs) or for the separation of Fc from Fab fragments. The binding characteristics of Protein A and G differ:
Protein A has a broad species reactivity. It binds well to IgG from human, rabbit, cow and guinea pig but weak to monoclonal antibodies.
Protein G has a broader species reactivity. It shows much stronger binding to IgG from mouse, rat and goat and also stronger binding to monoclonal antibodies.
Antibodies are eluted from the Protein A and G columns by low pH elution buffer.

Features

- Purification of monoclonal antibodies
- Selective purification of antibody fragments
- Purification or removal of polyclonal IgG from serum
- Separation of IgG sub-classes using a stepwise pH gradient
- Protein A for IgG from human (IgM, IgA, IgE, IgG_1, IgG_2, IgG_4), cat, dog, mouse, rabbit, pig, Guinea pig
- Protein G for IgG from human (IgG_1, IgG_2, IgG_3, IgG_4), cow, goat, horse, mouse (not IgM), pig, rabbit, rat and sheep

Protein	A	G
Human IgG_1	++	++
Human IgG_2	++	++
Human IgG_3	-	++
Human IgG_4	++	++
Human IgA	+	-
Human IgD	+	-
Human IgE	+	-
Human IgM	+	-
Mouse IgG_1	+	+
Mouse IgG_{2a}	++	++
Mouse IgG_{2b}	++	++
Mouse IgG_3	+	++
Mouse IgM	+/-	-
Rat IgG	++	++
Rat IgG1	+/-	+
Rat IgG2a	+/-	++
Rat IgG2b	+/-	+
Rat IgG2c	+/-	+
Rat IgM	+/-	-
Rabbit IgG	++	++
Hamster IgG	+	++
Guinea pig IgG	++	+
Bovine IgG	+	+
Sheep IgG	+/-	+
Goat IgG	+/-	+
Pig IgG	++	++
Chicken IgG	-	+/-
Fragments		
Human Fab	+	+
Human $F(ab')^2$	+	+
Human scFv	+	-
Human Fc	+	+
Human κ	-	-
Human λ	-	-

++ = strong affinity
+ = Moderate/slight affinity
+/- = Requires evaluation
- = No affinity

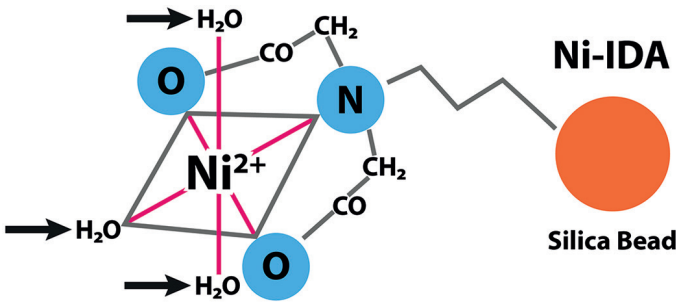
Order Information, Shipping & Storage

Order No.	Description	Amount
PR-PAK002	Protein A Starter Kit, 2 columns, buffers, 2 ultrafiltration spin columns and tubes	Kit
PR-PAK005	Protein A Kit with 5 columns	Kit
PR-PAK010	Protein A Kit with 10 columns	Kit
PR-PGK002	Protein G Starter Kit, 2 columns, buffers, 2 ultrafiltration spin columns and tubes	Kit
PR-PGK005	Protein G Kit with 5 columns	Kit
PR-PGK010	Protein G Kit with 10 columns	Kit
PR-PK001	Buffers for Protein A/G Kits for 10 applications with ultrafiltration spin filters and tubes	Kit

Shipped at RT; store at 4 °C

Ready-to-Use Ni-IDA Columns – First Choice for Higher Yields of His-tagged Proteins

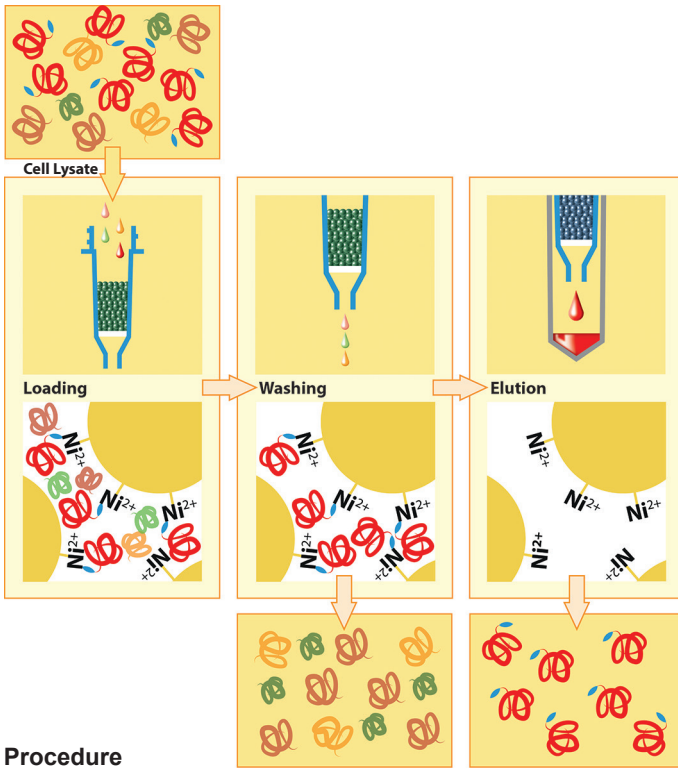
Ni-IDA Columns provide a fast and convenient routine tool for purification of recombinant polyhistidine-tagged proteins.
The form-stable silica matrix is precharged with Ni^{2+} ions and allows purification on the principle of Immobilized Metal Ion Affinity Chromatography (IMAC). Binding of proteins is based on the interaction between the polyhistidine tag of the recombinant protein and immobilized Ni^{2+} ions.



Silica-Based Ni-IDA Matrix
The Ni-IDA silica is an affinity chromatography matrix for purifying recombinant proteins carrying a His-tag. Histidine residues in the His-tag bind to the vacant positions in the coordination sphere of the immobilized nickel ions which enables strong and efficient binding of target protein. In free Ni-IDA the vacant positions are usually occupied by water molecules (as shown above).

Features

- Easy, fast, and cost-effective routine purification
- High binding capacity and high affinity
- Highest flexibility of purification:
  1. under native and denaturing conditions
  2. by gravity flow or by spinning
- Columns are **pre-packed** and **ready-to-use**
- Starting from diverse expression systems, e.g., *E. coli*, yeast, insect, and mammalian cells
- Universal use: suitable for small proteins, large protein complexes, proteins with low expression rates
- Improved target specificity by optimized silica-based Ni^{2+}-IDA matrix
- Imidazol-free loading and washing buffer
- Straightforward test of experimental protocols using His_6-GFP bacterial expression vector



Procedure
The purification of His-tagged proteins consists of 4 steps: cell lysis, loading & binding, washing, and elution. Cleared cell lysates are loaded onto the matrices. His-tagged proteins are bound, and other proteins pass through the matrix. After washing, His-tagged proteins are eluted with buffer from the matrix.

Simply replace your current Ni-NTA products, no optimization or protocol change necessary!

MobiSpin Ni-IDA Columns

Features

- For protein purification by spinning
- Very high binding capacity: up to 12 mg protein per spin column
- Excellent protein recovery rate of > 90%
- Simultaneous processing of multiple samples

MoBiTec Ni-IDA Columns

Features

- For protein purification by gravity flow
- Maximal binding capacity: up to 90 mg protein per column
- Protein recovery rate of > 80%
- Columns are long-term storable and reusable

Background

The chelating group of the Ni-IDA resin is based on IDA (iminodiacetic acid), which enables strong and efficient binding of target protein onto the IMAC matrix.
In contrast to traditional IDA matrices, MoBiTec Ni-IDA is an optimized matrix with low density of IDA ligands. This non-saturating surface concentration of IDA eliminates almost all non-specific interactions of contaminating host proteins with the adsorbent. As a result, MoBiTec Ni-IDA provides higher target protein purity.
IDA is a tridentate chelator which occupies three of the six binding sites in the coordination sphere of the Ni^{2+} ion. The remaining three coordination sites are usually occupied by water molecules and can be exchanged with histidine residues of the recombinant protein.

» Reliably test your experimental protocols by using MoBiTec's His_6-GFP bacterial expression vector. His_6-GFP protein - extracted from *E. coli* - can be detected by eye when loaded on, washed on, and eluted from the Ni-IDA columns. Every step can be quantified easily. «

Ask for Free Samples!

Order Information, Shipping and Storage

Order No.	Description	Amount
PR-HTK105	MobiSpin Ni-IDA Columns	5 Columns
PR-HTK110	MobiSpin Ni-IDA Columns	10 Columns
PR-HTK004	MoBiTec Ni-IDA Columns	4 Columns
PR-HTK010	MoBiTec Ni-IDA Columns	10 Columns
TOPO-HIS01	His_6-GFP Bacterial Expression Vector	10 µg DNA
PEG01*	pEG-His1 Vector	5 µg DNA

Columns contain dry matrix; shipped at RT; storable at RT for at least 1 year.

*Convenient cloning and subsequent purification of your protein of interest: pEG-His1 vector for IPTG inducible expression of gene products with C-terminal RGS motif and His_6-tag. 5' and 3' primers for sequencing are provided in addition.

Chapter V

Compact Reaction Columns (CRC) Mobicols with Enzymatically Active Matrices

CRC with Immobilized Proteases

CRC with Immobilized Nucleases

CRC with other Immobilized Enzymes

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 ≥103 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.

Procedure

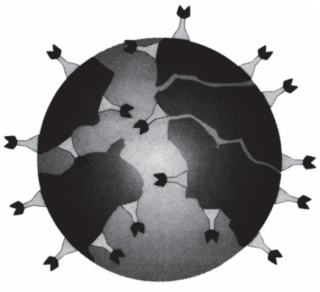
- Equilibrate column with reaction buffer by flow-through (since column is provided with storage buffer).
- Load substrate solution in reaction buffer.
- Incubate.
- Elute product solution by:
  a) centrifugation (smaller volumes, matrix G3m)
  b) continuous flow mode (larger volumes, matrix F7m)

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.



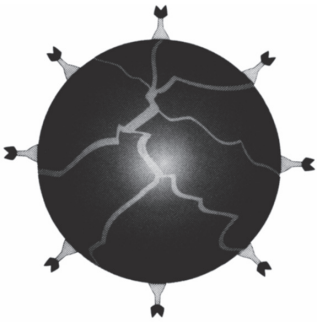
F7m
Large pores-
high activity!

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.

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.



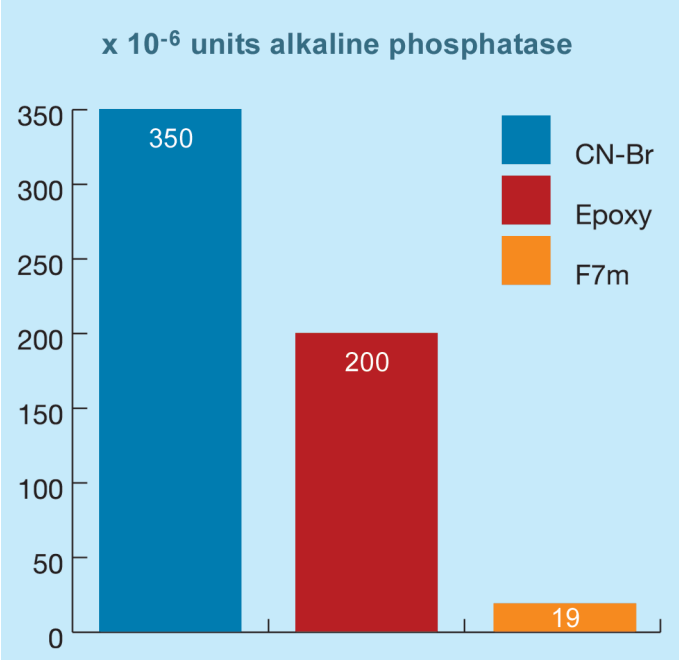
G3m
Small pores-
no sample dilution!

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

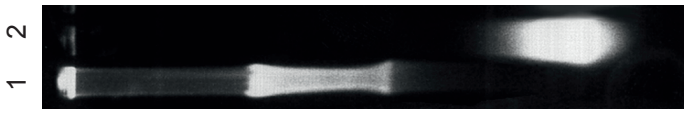
DNA plasmids are biologically as active (in transformation) after passage through a CR-column (Proteinase K, RNase A) as the untreated molecules.

Applications

Removal of genomic DNA

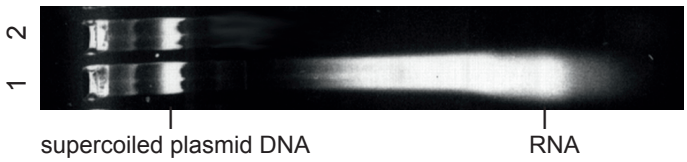
Immobilized DNase I on G3m matrix is a useful tool for the removal of contaminating genomic DNA during RNA purification for single-cell cDNA-PCR: Standard DNase I digestion of RNA prepared from a single cell often leads to loss of material due to the required extraction step. Therefore, a method eliminating 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.

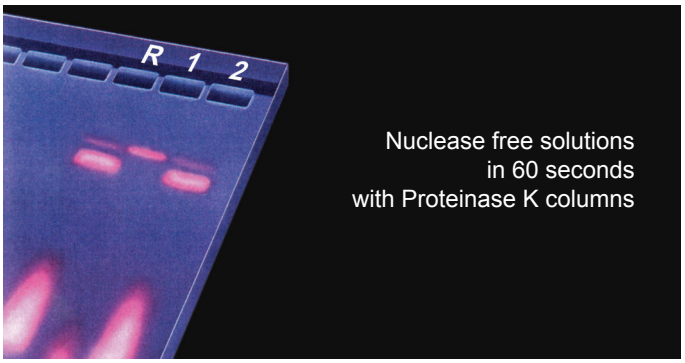
Easy digesting RNA during plasmid preps without phenol extractions



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.

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!

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.

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

www.mobitec.com

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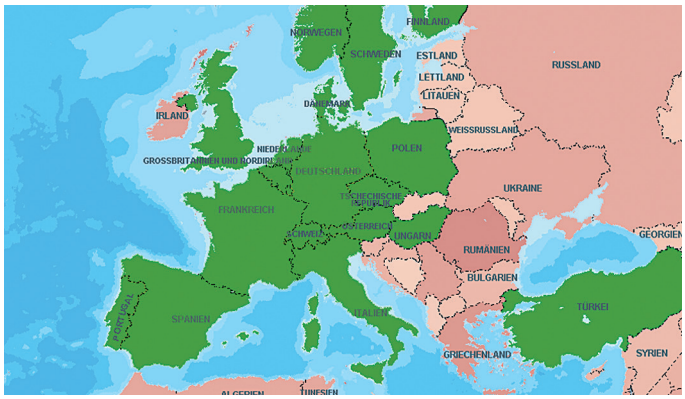

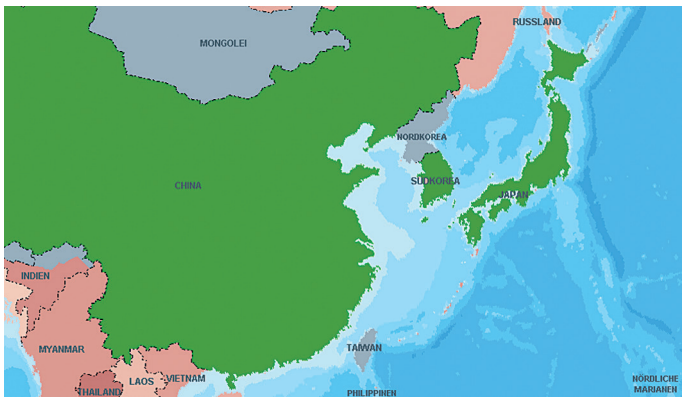

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