MobiSpin S-Columns

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Contents

1. Features

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.

- Compatible with laboratory standard
- Columns are pre-packed, equilibrated, and ready to use
- Very fast procedure: sample purification in less than 4 minutes
- Easy handling: spin, load sample, spin, and collect the purified product
- Reproducible results with simplified protocols
- Numerous samples can be processed simultaneously
- No sample dilution
- Large number of applications, e.g.,
    - buffer exchange,
    - nucleotide removal,
    - plasmid, oligo, and PCR reaction purification
    - removal of unincorporated dyes or dye terminators

2. Introduction

MobiSpin S-Columns pre-packed with Sephacryl® HR matrices are designed for a wide variety of separation tasks such as 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, do enter the pores of the matrix beads and are held back. A detailed overview on the functional principle is given in figure 1.

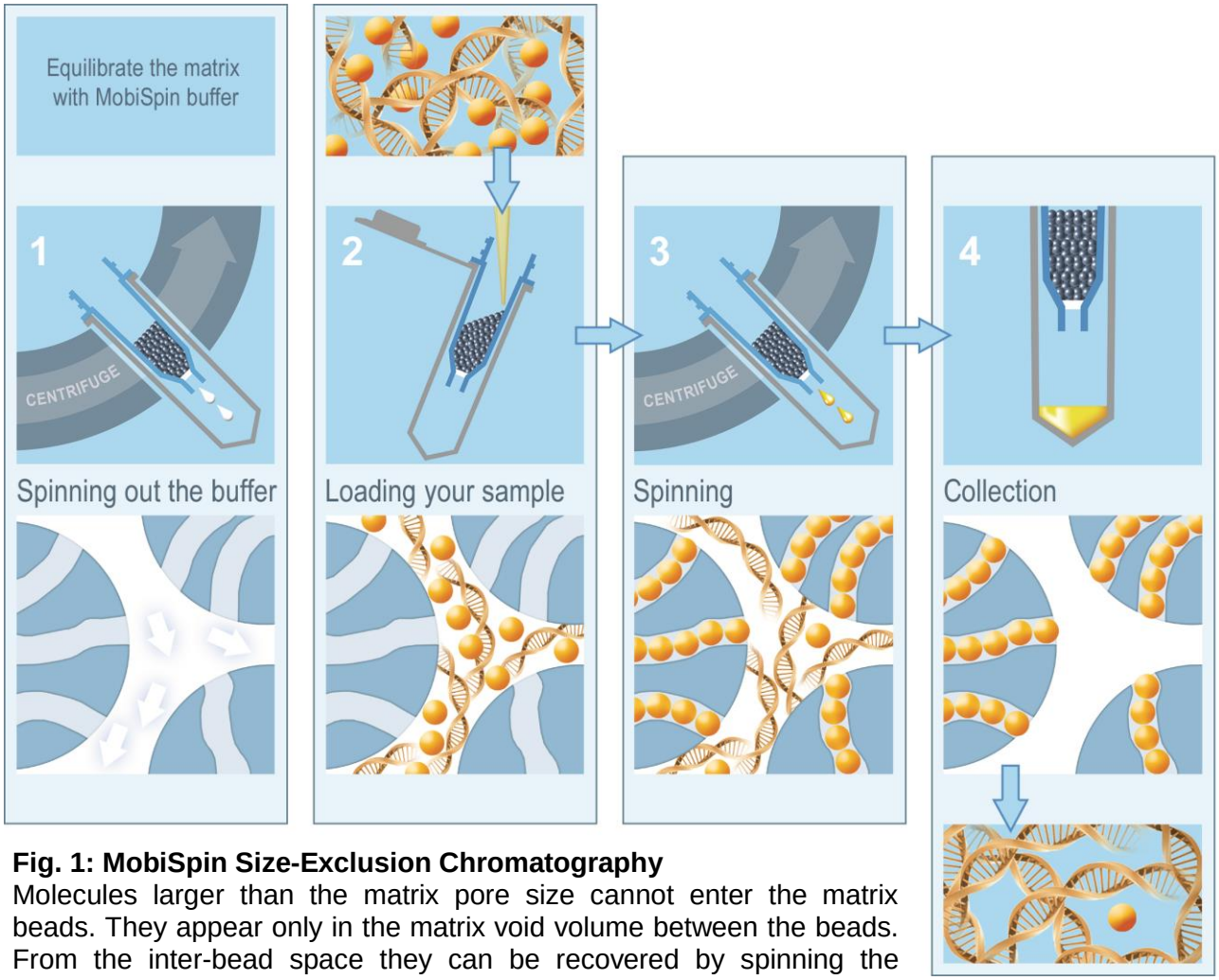

Fig. 1: MobiSpin Size-Exclusion Chromatography
Molecules larger than the matrix pore size cannot enter the matrix beads. They appear only in the matrix void volume between the beads. From the inter-bead space they can be recovered by spinning the column in a collection tube in a benchtop centrifuge. Strong spinning elutes the column void volume without dilution. Molecules smaller than the matrix pore size and hydrated salt ions located inside the matrix beads are not eluted by spinning.

3. Product contents

3.1. MobiSpin S-Columns - Contents

Order#	Product	Components included
SCO200	MobiSpin S-200 Columns	20 columns, pre-packed with Sephacryl® S-200 HR resin, equilibrated in MobiSpin Buffer (10 mM Tris/HCl pH 7.6; 1 mM EDTA)
SCO300	MobiSpin S-300 Columns	20 columns, pre-packed with Sephacryl® S-300 HR resin, equilibrated in MobiSpin Buffer (10 mM Tris/HCl pH 7.6; 1 mM EDTA)
SCO400	MobiSpin S-400 Columns	20 columns, pre-packed with Sephacryl® S-400 HR resin, equilibrated in MobiSpin Buffer (10 mM Tris/HCl pH 7.6; 1 mM EDTA)

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

MobiSpin S-200 Columns: Red cap and label
MobiSpin S-300 Columns: Green cap and label
MobiSpin S-400 Columns: Yellow cap and label

Fig.2: **MobiSpin S-Columns** with color-coded caps
for easy identification (red: S-200, green: S-300, yellow: S-400)

3.2. MobiSpin S-Columns - Constituent parts

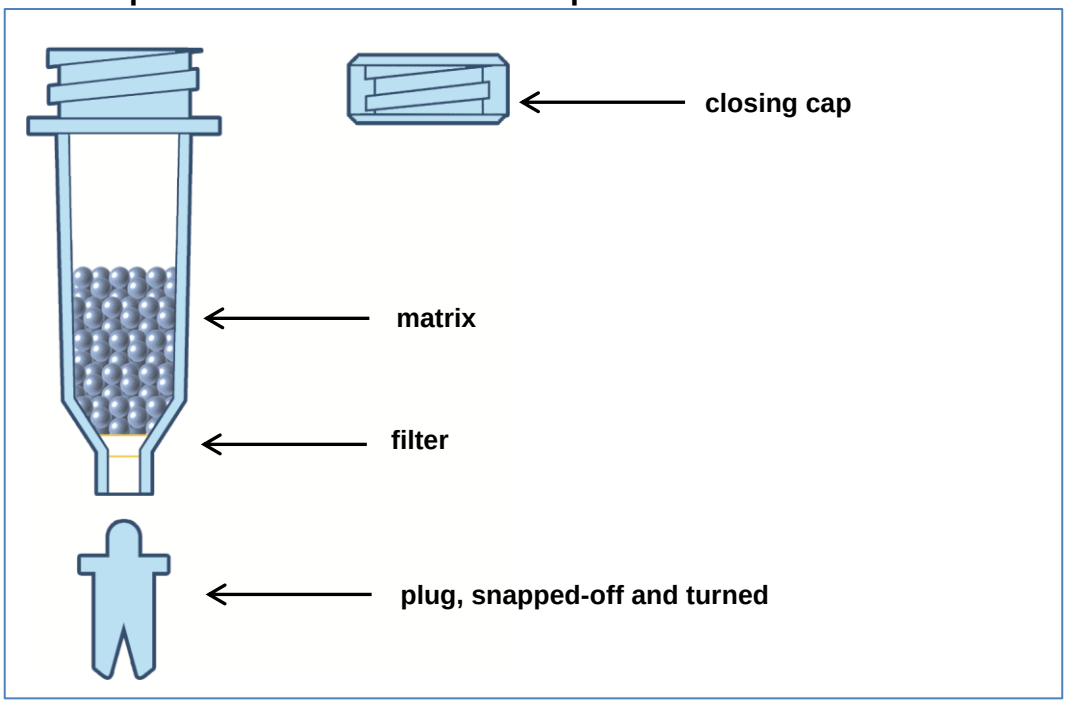


Fig. 3: MobiSpin S-Column
The MobiSpin S-Column is closed with a screw cap and contains the Sephacryl® HR matrix equilibrated with MobiSpin Buffer. The tip of the MobiSpin Column is a fixed snap-off plug. Prior to the first centrifugation step it has to be bent down and removed. The plug can be reused for closing by turning it upside down.

3.3. Equipment and materials to be supplied by user

- Microliter pipettes
- Standard microcentrifuge for 1.5 ml and 2 ml microcentrifuge tubes
- Microcentrifuge tubes 1.5 ml or 2 ml (as actual sizes differ, please check suitability in advance)

3.4. Storage and expiry

MobiSpin S-Columns are storable at 4 °C for at least one year.

4. Terms and Conditions

For research use only. NOT recommended or intended for diagnosis of disease in humans or animals. Do NOT USE internally or externally in humans or animals. All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses, and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water.

Product warranty is limited to our liability to replacement of this product. All other warranties, expressed or implied, including but not limited to any implied warranties of merchantability or fitness for a particular purpose, are excluded and do not apply. We shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

5. Technical Information

5.1. General information

When used in a spin column format, gel filtration resins do not show a fixed exclusion limit. This is only meaningful in continuous flow processes where molecules (being purified via gel filtration resins) have sufficient time to reach equilibrium between the time spent in the pores of the gel matrix and the time spent in the eluent stream.

Using spin-column chromatography, the observed exclusion properties allow the product to pass through the gel while smaller sample impurities are retained. This 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.

The MobiSpin Columns are designed for a wide variety of nucleic acid purification applications. When choosing the appropriate column for a particular application, a few issues need to be considered to determine the resin which is best suited for that application. Specifically, an estimate must be made of the size of the impurities in the sample relative to the size of the product being isolated. The compromise between sample purity and product yield must also be considered. Please refer to the “Sample Volume Guide” on page 10 to determine the conditions which are recommended for each specific application. Some general guidelines to follow when determining the appropriate purification conditions are discussed below.

5.2. Column usage guidelines

- 20 x 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.
- Purity versus yield: since purity is generally inversely proportional to yield, larger sample volumes will give higher yields but lower purities, and vice versa. Therefore, a general rule for any particular volume is: the larger the pores size of the resin, the greater the purity and the lower the yield of the resulting product. Gel filtration matrices with higher 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. For each resin type there is a uniform proportional loss of sample due to the nature of the process.

- Retention: for a given sample volume, product retention inversely correlates to molecular size. As the size of the product increases, its relative retention decreases.
- Optimizing: in general, we recommend loading volumes of 25-100 µl for all applications. For occasions in which the current sample volume is different from those recommended in the Sample Volume Guide on page 10, we recommend the following sample adjustments:
  1) For large sample volumes, apply an aliquot of the sample to the column. More than one column can be used if the total sample volume exceeds the recommended sample volume for a particular application.
  2) For small sample volumes, dilute the sample to a larger volume to increase product recovery.
  3) Precipitate the sample and redissolve in an appropriate volume.

6. Standard Protocol

- Resuspend the resin in the column by vortexing.
- Bend off the tip of the column and loose the cap one fourth turn.
- Place the column in a 1.5 ml or 2 ml microcentrifuge tube.
- Pre-spin the column 1 minute at 800 x g in a microcentrifuge with a fixed-angel rotor.

Note: before using a MobiSpin Column, it may be of significant importance to calculate the speed at which the column should be centrifuged. The calculation of the appropriate centrifugation speed ensues from the following formula:

$RCF = (1.12) \times (r) \times (rpm/1000)^2$,

whereby RCF is the relative centrifugal force, r the radius measured in mm from the center of the spindle to bottom of rotor bucket, and rpm the revolutions per minute.

Example:
for a force of 800 g the above equation resolves to

$rpm = 1000 \sqrt{714/r}$

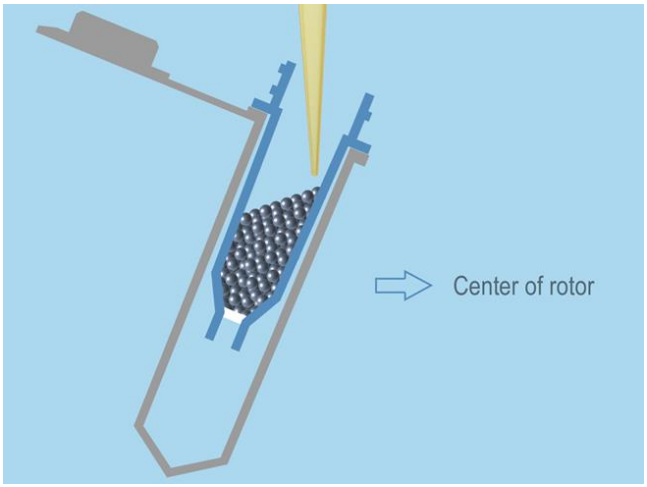
r = radius in mm measured from center of spindle to bottom of rotor bucket

rpm = revolutions per minute

With a rotor having a radius of 110 mm, the appropriate speed would be 2548 rpm.

- Use the column **immediately** after removing the equilibration buffer from the resin to avoid drying up!
- Place the column in a new 1.5 ml tube and slowly apply the sample (10-100 µl) to the upper side of the slanted matrix surface as shown in Fig. 4. Take care 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.

Fig. 4: Load the sample onto the higher side of the slanted matrix surface. For subsequent centrifugation the column should be placed with the higher side of the slanted matrix toward the center of the rotor.



7. Sample Volume Guide

			S-200 (10 µl) S-300 (10 µl) S-400 (25-50 µl)	**25-mers**
	S-300 (25-50 µl) S-400 (50-100 µl)	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)	**18-mers**
S-200 (25-50 µl) S-300 (25-50 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) S-400 (50-100 µl)	**8-mers**
S-200 (25-50 µl) S-300 (25-50 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) S-400 (50-100 µl)	**NTPs/salts**
>20-mers	**>50-mers**	**>200-mers**	**>500-mers**	

Maximum Contamination Size

Minimum Product Size

Table 1: Sample Volume Guide
Sample volumes 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). Example: to separate an 8-mer contaminant from a 100 bp molecule, check the table at the 8-mers row and the >50-mers column. Dependent on your sample volume select S-200 (if your sample volume is 25-50 µl), S-300 (if you sample volume is 25-75 µl), or S-400 (if your sample volume is 50-100 µl). This table is intended only as a general guideline. Personal experience and judgement should be used when making the final selection of matrix type for your particular application. When in doubt, apply a sample volume of 50 µl.

8. Applications

We recommend MobiSpin S-Columns as rapid and efficient tool for the following applications:

- **Removal of dNTPs, oligos, and salt**
- **Removal of dye terminators** or unincorporated **labeled nucleotides** from DNA labeling reactions

Removal of dNTPs, oligos, and salt

MobiSpin S-Columns pre-packed with **S-200, S-300, or S-400 Sephacryl® HR** matrix 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. For more specific columns selection, the size of the product (DNA/RNA), the length of the nucleic acid contaminant, and the sample volume must be considered (see Table 1, p. 10).

Removal of dye terminators or unincorporated labeled nucleotides

MobiSpin S-Columns pre-packed with **S-200, S-300, or S-400 Sephacryl® HR** matrix are particularly suited for the

- removal of fluorescent dye dideoxyterminators (e.g., Cy5/Cy3 nucleotides) from cycle sequencing reactions.
- removal 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. For choosing the most applicable Sephacryl® HR matrix the exact size of the labeled DNA and the sample volume has to be considered (see Table 1, p. 10).

9. Order Information, Shipping, and Storage

Order#	Product	Amount
SCO200	MobiSpin S-200	20 columns
SCO210	MobiSpin S-200	100 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
SCO234	MobiSpin S-200, S-300, S-400	3 x 10 columns
shipped at RT; store at 4 °C		

The columns are pre-packed with resin and equilibrated in MobiSpin Buffer (10 mM Tris/HCl pH 7.6, 1 mM EDTA). Store the columns at 4°C.

10. Related Products

Order#	Product	Amount
SCO500	MobiSpin G-50	20 columns
SCO510	MobiSpin G-50	100 columns
SCO100	Desalting MobiSpin Columns	20 columns
SCO110	Desalting MobiSpin Columns	100 columns

11. Contact and Support

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