MobiSpin - Columns for DNA Purification

Product Information and Instructions
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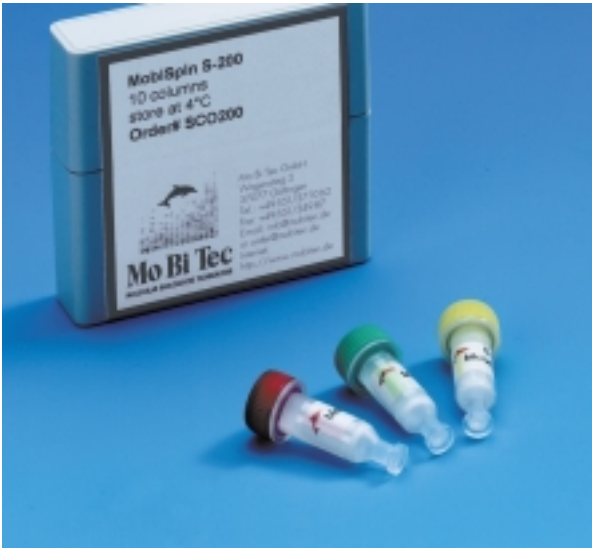
Mo Bi Tec
MOLECULAR BIOLOGISCHE TECHNOLOGIE

MobiSpin Columns

- for buffer exchange
- nucleotide removal
- plasmid, oligo and PCR product purification



Content

1. Advantages:

Spin column chromatography offers many advantages over traditional liquid chromatography:

- compatible with laboratory standard
- no column packing, column comes pre-packed and equilibrated
- easy handling: spin, load sample, spin, and collect the purified product
- no sample dilution
- reproducible results with simplified protocols
- one application in less than 4 minutes
- numerous samples can be processed simultaneously
- large number of applications

2. Components

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

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

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3. Background

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 an 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 8 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.

4. 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.

- 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 pore 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 nano gram 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 8, 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.

5. 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 (e.g. Sarstedt tube) for support. Alternatively, cut the cap from a flip-top tube and use this as a support.
- 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.

- Place the column in a new 1.5 ml tube and slowly apply the sample (10 - 100 µl) to the top-center 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.

Centrifuges:

- Heraeus Biofuge 13 with an 18-position fixed-angel rotor use at 3000 rpm
- Eppendorf 5415C with an 18-position fixed-angel rotor use at 3000 rpm
- If you do not have a variable-speed microcentrifuge, you may use a single-speed microcentrifuge. This may result in a small loss of column performance.

This table represents the sample volumes which have been tested for each resin and which will provide an acceptable level of performance for a given

6. 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 Contaminant Size

Minimum Product Size

application. The box in the table which represents the intersection of the minimum product size and estimated maximum contaminant size lists the resins which are appropriate for that application and the recommended range of sample volumes. This table is intended only as a general guideline. Personal experience and judgement should be used when making the final selection of column type for your particular application. When in doubt, apply a sample volume of 50 µl.

7. Examples of applications

1. DNA Purification Prior to Sequencing

In this experiment, plasmid DNA was prepared using a standard alkaline lysis procedur. 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. All three types of MobiSpin columns yielded results comparable to the CsCl-purified template DNA.

2. 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 the standard protocol (page 7). For comparison, a 500 µl aliquot of the same deprotection solution was purified on a Sephadex G-25 DNA Grade drip column. The quality of DNA obtained with the MobiSpin Column analyzed by electrophoresis was equivalent to that obtained with the drip column. Purification using the MobiSpin column took only one-fifth of the time required to purify the sample on the drip column.

3. Loading Levels

Using our MobiSpin columns, DNA can be recovered over a wide range of sample loading levels.
In our control experiment, 1 ng of labeled pUC18 DNA in 50 µl sample volume was purified over each of the MobiSpin column types S-200, S-300 and S-400 in the absence of any carrier DNA. The complete removal of un-incorporated, labeled nucleotides was achieved with excellent recovery of labeled DNA. In a second test, various amounts of labeled DNA (25 - 250 ng) from an end-labeling reaction were combined with large quantities of unlabeled DNA (25 - 250 ng). The mixtures were purified over each type of MobiSpin column. The test showed, that under these conditions regardless the resin type, the presence of relatively large amounts of unlabeled DNA had virtually no effect on the yield of labeled DNA or on the ability of the column to remove labeled, un-incorporated nucleotides from the reaction mixture.

4. 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, buffer exchange and nucleotide removal were achieved using a MobiSpin S-200 column (1. 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 (2. step). Finally, the purified, single-stranded DNA was sequenced directly.

5. Removal of ^{32}P-labeled Nucleotides

MobiSpin columns can be used to remove large amounts of un-incorporated radiolabeled nucleotides. In our experimental set up, a 792 bp end-labeled fragment was purified over each of the three MobiSpin column types (S-200, S-300, S-400). The un-incorporated 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 in oligolabeling reactions. The un-incorporated, labeled nucleotides from 50 µCi reactions were completely and efficiently removed during purification of the reaction products on each type of MobiSpin column.

8. Order Information

order #	description	amount
SC0200	MobiSpin S-200 (Sephacryl® S-200 HR)	20 columns
SC0300	MobiSpin S-300 (Sephacryl® S-300 HR)	20 columns
SC0400	MobiSpin S-400 (Sephacryl® S-400 HR)	20 columns
SC0234	MobiSpin S-200, S-300, S-400	3 x 10 columns

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

Next to our three different Sepharcryl® resins a **new MobiSpin column with Sephadex® G-50 resin** is available (for purification of DNA after labeling reactions, i.e. removal of un-incorporated dyes or dye terminators).