Desalting MobiSpin Columns

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Content

1. Features

Desalting MobiSpin Columns are developed for rapid and efficient routine purification of nucleic acids from smaller contaminants as small as NaCl. In comparison to traditional liquid chromatography the Desalting MobiSpin Column chromatography offers numerous advantages:

- Compatibility with laboratory standard
- Columns are pre-packed and long-term storable at room temperature
- Easy handling: 1. Equilibrate with MobiSpin Buffer, spin, and discard the flow through
  2. Load sample, spin, and collect the purified product
- Desalting efficiency > 90%
- Improved recovery of small-sized nucleic acid fragments
- No sample dilution
- Reproducible results with simplified protocols
- Sample loading and recovery in less than 3 minutes
- Numerous samples can be processed simultaneously

2. Description

Spin column chromatography combines effectiveness of gel filtration with speed of centrifugation. The novel silica resin of the Desalting MobiSpin Column is comprised of uniform microscopic beads of a porous, form-stable material. It allows the selective removal of molecules as small as NaCl. Since this new matrix material works without moisture expansion (contrasting sepharcryl and sepharose resins, which swell in buffer), resins are allowed to fall dry without any loss in purification efficiency. Therefore, Desalting MobiSpin Columns give reproducible results of high quality and are much easier to handle. Furthermore, the columns are long-term storable in dry state.

The new matrix works on the proven principle of size-exclusion. Nucleic acid molecules larger than the pore size are excluded from the resin. These molecules quickly move 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. Thus, nucleic acid molecules are eluted from the column in order of decreasing molecular size. A detailed overview on the functional principle is given in Fig.1.

After adding MobiSpin Buffer for equilibration, the Desalting MobiSpin Columns are ready to use for rapid and efficient purification of nucleic acids using a microcentrifuge. Nucleic acids purified with these columns are well suited for use in many molecular biology experimental procedures, including sequencing, labeling, PCR, cloning, and *in vitro* transcription (Sambrook & Russell, 2001).The separation performance of the Desalting MobiSpin Columns has been vigorously tested with a wide range of nucleic acid fragments. In particular, fragments of 100 bp or smaller showed an improved recovery rate in comparison to conventional sepharcyl spin columns. For instance, with 75 bp sized fragments more than 20% higher recovery (measured in relation to the starting amount) is attained (see 6.2., page 8).

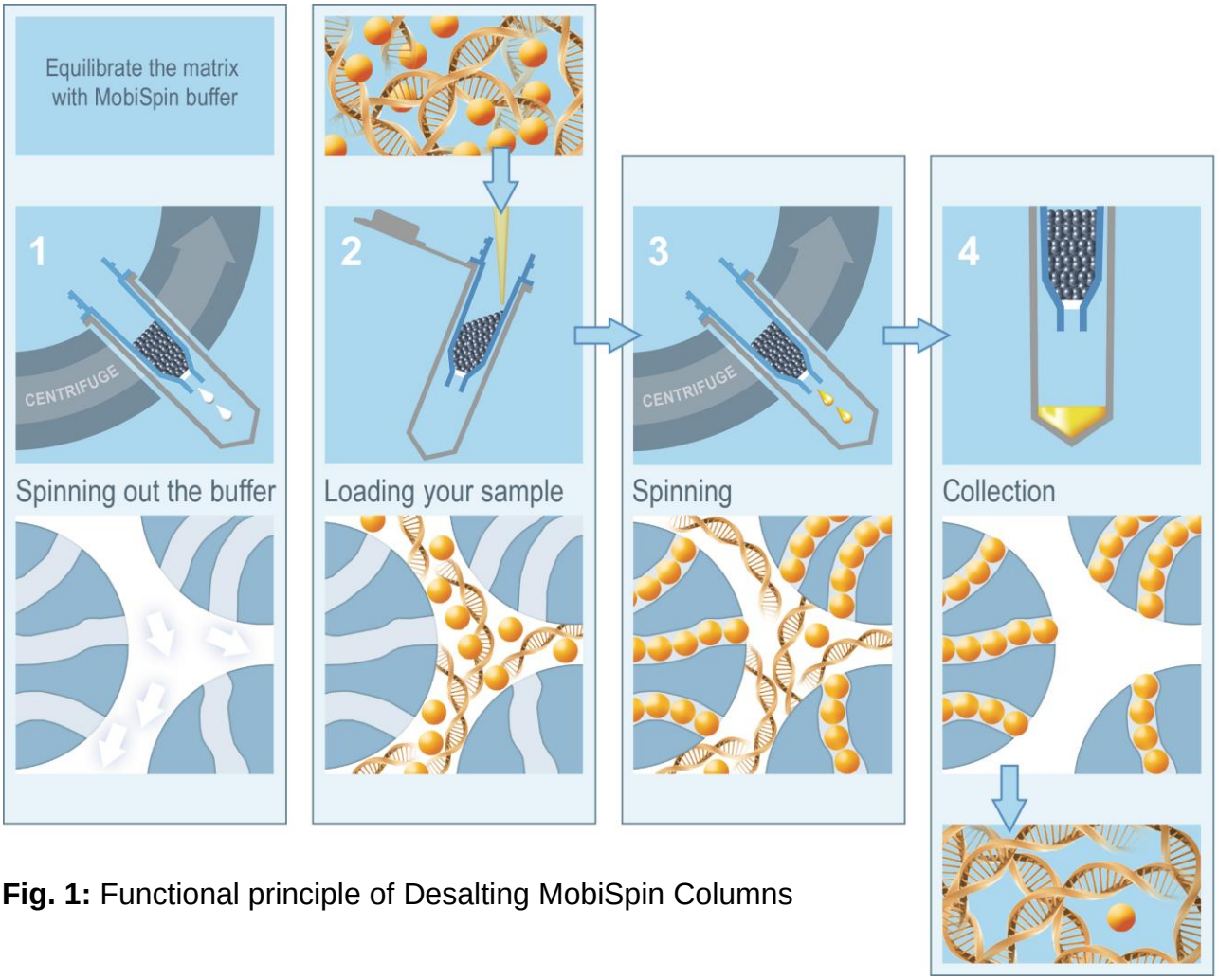


Fig. 1: Functional principle of Desalting MobiSpin Columns

3. Product Contents

3.1. Kit components

Order#	Product	Components included
SCO100	Desalting MobiSpin Columns (20 purifications)	20 Columns with matrix (dry) prepacked; 2 ml MobiSpin Buffer 10x (100 mM Tris/HCl pH7.6; 10 mM EDTA); user manual
SCO110	Desalting MobiSpin Columns (100 purifications)	100 Columns with matrix (dry) prepacked; 10 ml MobiSpin Buffer 10x (100 mM Tris/HCl pH7.6; 10 mM EDTA); user manual

3.2. Desalting MobiSpin Column – constituent parts

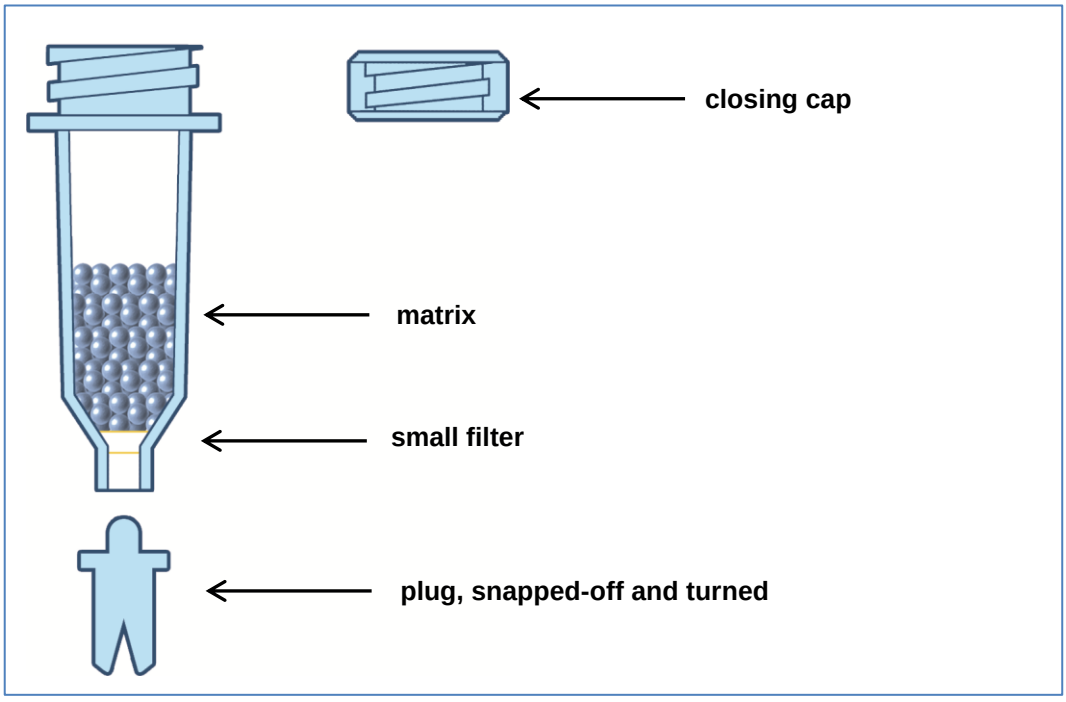


Fig. 2: Desalting MobiSpin Column
The Desalting MobiSpin Column is closed with a screw cap and contains the new matrix comprised of uniform microscopic beads of a porous form-stable silica material. The matrix is held back by a 35 µm filter. The tip of the MobiSpin Column is a fixed snap-off plug. Before 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)

4. Terms and Conditions

4.1. Safety warnings precautions and product warranty

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 (See Material Safety Data Sheet(s) and/or Safety Statement(s) for specific recommendations).
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.

4.2. Storage

Store MobiSpin Columns at room temperature and MobiSpin Buffer at 4 °C.

4.3. Expiry

MobiSpin Columns: at least 1 year at room temperature
MobiSpin Buffer (10x): 1 year at 4 °C

5. Technical Information

5.1. Loading volumes

Load 20-50 µl onto a column for all applications. For larger sample volumes, either use more than one column or reduce the sample volume by drying or precipitation. For smaller sample volumes, dilute the sample to improve product recovery. If the volume recommendations are followed, the yield of purified DNA is expected to be 60-80%.

5.2. Desalting capacity

30 µl of 500 mM NaCl solution can be desalted > 90% with Desalting MobiSpin Columns. For probes with a much higher total quantity of salt, we recommend performing a second purification step with an additional Desalting MobiSpin Column.

5.3. Nucleic acid purification criteria

Concentration and quantity

Nucleic acid concentrations of a wide range can be applied to the Desalting MobiSpin Columns (50 ng/µl to 1 µg/µl). Critical for a good recovery rate is not the concentration itself but the total quantity of loaded nucleic acids. Starting with high overall amounts will result in higher recovery rates of nucleic acids. By applying 3-25 µg DNA fragments, a recovery rate of 60-80%, resp., has been achieved.

Size exclusion limits

Lower limit: DNA fragments with a size of 35 bp or more can be purified with a recovery rate of > 60%. Even smaller fragments can be recovered. The recovery rate is strongly dependent on the starting concentration (see above, “Concentration and quantity”).

Upper limit: Based on the functional principle of the Desalting MobiSpin Columns, larger nucleic acid molecules are easier to recover. DNA fragments up to 5 kb have been purified with a recovery rate of > 80%.

5.4. Centrifugation notes

Centrifuge type

Use a standard benchtop microcentrifuge with a centrifugal force of 2500 x g (equation for g-force calculation see below).

Microcentrifuge tubes

MobiSpin Columns fit in 1.5 ml and 2.0 ml microcentrifuge tubes. Use the 2.0 ml microcentrifuge tubes for the initial column equilibration step and 1.5 ml microcentrifuge tubes for sample collection during centrifugation.

g-force calculation

Benchtop microcentrifuges capable of generating a minimum force of 2500 x g are suitable. The gravitational force created at a particular rpm (revolutions per minute) is a function of the radius of the microcentrifuge rotor. Consult the microcentrifuge instruction manual for conversion information from rpm to g-force. Alternatively, to calculate the speed (rpm) required to reach a gravitational force of 2500 x g, use the following equation:

$$RCF\ (g) = (1.12 \times 10^{-5})\ (rpm)\ 2r$$

r: radius (cm), measured from the center of the rotor to the middle of the MobiSpin Column.

6. Application Notes

6.1. Separation performance of MobiSpin Columns: desalting DNA molecules

The separation performance of Desalting MobiSpin Column has been extensively tested with a wide range of nucleic acid fragments. The recovery rate and the level of desalting are depending on the probes parameters:

a) amount and size of nucleic acids
b) amount of salt.

The following table gives an overview on DNA purification (level of desalting and recovery of DNA) in consideration of size and amount of the loaded fragments. In each case, a volume of 30 µl was loaded onto the column, containing 500 mM NaCl.

Tab. 1: DNA purification in consideration of size and amount of loaded fragments

Size of DNA	Amount of DNA	Recovery	Level of desalting
2000-5000 bp	25 µg	> 80%	> 90%
2000-5000 bp	3 µg	> 60%	> 90%
75-100 bp	15 µg	> 70%	> 90%
35-50 bp	15 µg	> 60%	> 90%

DNA recovery was determined by measuring the DNA concentrations before and after MobiSpin Column purification by two independent methods of measurement:

1. Absorbance measurement at 260 nm and 280 nm and calculation of DNA amount according to the Beer-Lambert law.
2. Separation by polyacrylamide gel electrophoresis followed by software based calculation of band intensity. Please consider: estimation of DNA bands separated by agarose gel electrophoresis resulted in underestimated values of recovery.

6.2. Desalting MobiSpin Columns versus conventional spin columns

To investigate purification and recovery efficiency of the Desalting MobiSpin Columns in comparison to conventional desalting spin columns with a sephacryl matrix, several tests with both kinds of columns were performed. In all cases a desalting efficiency of > 90% could be achieved. Whereas the recovery rates of large-sized fragments > 2kb were nearly comparable (about 80%), the Desalting MobiSpin Columns showed considerably improved recovery rates with small-sized fragments (Fig. 3).

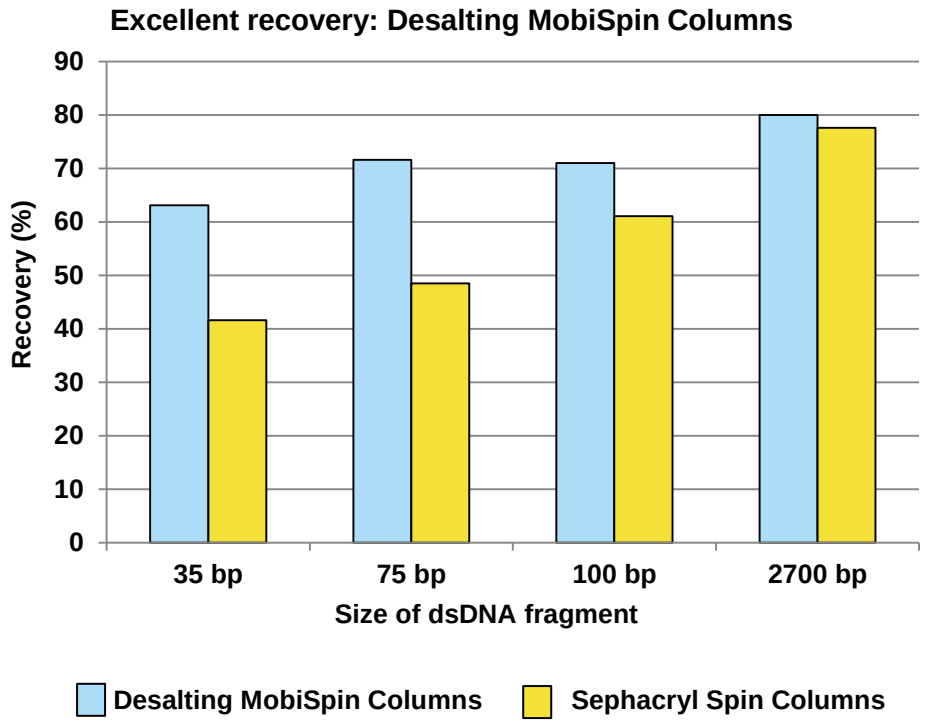


Fig. 3: Improved recovery efficiency with small-sized dsDNA fragments. Recovery rates of the Desalting MobiSpin Columns in comparison to conventional desalting spin columns with sepharcyl matrix were calculated according to the following methods: **Recovery rates of small-sized fragments (≤100 bp**) were tested by loading 15 µg of a defined DNA fragment mixture onto each kind of column. DNA fragments (before and after purification) were separated by polyacrylamide gel electrophoresis and band intensity of each fragment was calculated with appropriate software. **Recovery rates of a 2300 bp fragment** were calculated photometrically by determining concentrations before and after purification. In this case 25 µg have been loaded onto the respective columns.

7. Standard Protocol

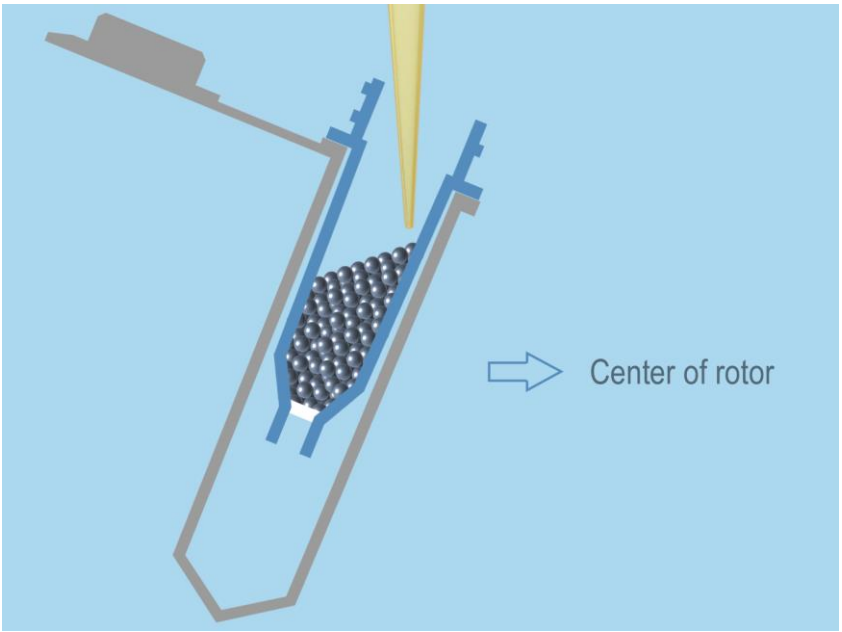
7.1. Equilibration of Desalting MobiSpin Columns

- Dilute the 10x concentrated MobiSpin Buffer stock solution to 1x concentration.
- Tap the Desalting MobiSpin Column slightly onto the desk to remove entrapped air from the matrix.
- Carefully pipette 500 µl 1x MobiSpin Buffer into the column.
- Pipette the matrix/buffer solution three times up and down for equilibration and let the matrix settle down for 10 minutes.
- Carefully discard excessive buffer on the top of the matrix by pipetting.
- Bend off the tip of the column and place the column into a 2.0 ml microcentrifuge tube.
- Centrifuge the column (without cap) for 2 minutes in a microcentrifuge at 2500 x g to completely remove the remaining MobiSpin Buffer. The flow-through is discarded.

7.2. Sample purification

- Place the column into a clean 1.5 or 2.0 ml microcentrifuge tube.
- Carefully apply the sample to the upper side of the slanted matrix surface as shown in Fig. 4.
- Place the loaded column together with the tube into a microcentrifuge. The column should be positioned with the higher side of the slanted matrix facing the center of the rotor. Centrifuge the column for 2 minutes at 2500 x g. The purified DNA is collected in the microcentrifuge 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.



8. Order Information, Shipping and Storage

Order#	Product	Quantity
SCO100	Desalting MobiSpin Columns (20 purifications)	1 kit
SCO110	Desalting MobiSpin Columns (100 purifications)	1 kit
shipped at RT; store columns at RT and buffer at 4 °C		

9. Contact and Support

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