SPIN COLUMNS

Product Numbers **S 1295, S 2045, S 2170, S 1420, S 0670, S 2295, S 0795, S 1545, S 3170, S 1670, S 0920, S 2420, S 3295, S 1045, S 1795, S 1170, and S 2670**

ProductInformation

TECHNICAL BULLETIN

Product Description

Spin Columns combine the economy and effectiveness of gel filtration with the speed and simplicity of centrifugation. They are designed to selectively remove contaminating molecules based on size. The spin columns are offered in six gel matrix pore sizes to cover a wide range of applications. The highest sample recovery is observed when the molecule of interest is significantly larger than the spin column pore size. Conversely, the highest purity is achieved when the contaminants are significantly smaller than the spin column pore size. Procedures are provided for standard and large capacity column purification. The Spin Columns are supplied in TE, STE, or DEPC-treated water, but can be re-equilibrated with a buffer of choice. All spin columns are autoclaved to inactivate nucleases. Columns supplied in DEPC-treated water must be equilibrated in a suitable buffer for maximum recovery and purification. Directions are provided for buffer replacement.

It is recommended that a swinging bucket centrifuge be used in these procedures to allow the sample to pass through the column uniformly. The sample may by-pass the column matrix when a fixed-angle rotor is used, resulting in incomplete or inconsistent purification. For easy handling, columns and fitted 2 ml collection tubes can be placed inside a 17 x 100 mm polypropylene tube before centrifugation.

Table 1.
Expected Recovery from Spin Columns

Column	Product Number	Expected Recovery
Spin Column 10	S 1295, S 2045	Recover ≥ 80% of molecules 26 base pairs or larger
Spin Column 30	S 2170, S 1420, S 0670	Recover ≥ 80% of molecules 76 base pairs or larger
Spin Column 100	S 2295, S 0795, S 1545	Recover ≥ 80% of molecules 194 base pairs or larger
Spin Column 200	S 3170, S 1670, S 0920, S 2420	Recover ≥ 80% of molecules 204 base pairs or larger
Spin Column 400	S 3295, S 1045, S 1795	Recover ≥ 80% of molecules 872 base pairs or larger
Spin Column 1000	S 1170, S 2670	Recover 50-80% of molecules 2,000 base pairs or larger

Precautions and Disclaimer

Spin columns are for laboratory use only; not for drug, household, or other uses. Warning statements are included on the label or in the components section of this bulletin where applicable. In addition, when radioactive tracers are used, standard procedures for safely handling radioactive materials should be followed.

Storage/Stability

Store at room temperature.

Procedure

For efficient recovery of the target molecules, the lower limit of the sample volume should be used (See Table 2). If the sample volume is less than the recommended range, add sufficient buffer to bring the volume up to the lower limit before loading on to the column. If the maximum recommended sample volume is exceeded, the trailing edge of the desired eluate can be contaminated with the leading edge of the smaller, obstructed molecules. Per cent recovery can be increased by centrifuging for a longer period of time; however, this may decrease the purity of the recovered DNA.

If the small nucleic acid fragments to be removed from the sample are complementary to the sample or include complementary sequences (for example PCR primers and template), denature the sample by heating in a boiling water bath for ten minutes, followed by cooling on ice. This will denature complementary sequences and result in a more effective purification.

Columns supplied in DEPC-treated water must be equilibrated (see Buffer Replacement Procedure) in a buffer to prevent the sample from binding to the column matrix. We recommend using STE and TE buffer for nucleic acid purifications. Other buffers may be used; however, their effectiveness must be determined empirically.

Table 2.
Recommended Sample Volumes

Column Type	Standard Sample Volume Range	Large Capacity Volume Range
Spin Column 10 and 30	25-50 µl	50-120 µl
Spin Column 100	40-75 µl	75-150 µl
Spin Column 200	40-75 µl	100-200 µl
Spin Column 400	40-75 µl	150-250 µl
Spin Column 1000	70-100 µl	250-350 µl

Procedure

1. Invert the spin column several times to suspend the matrix uniformly.
2. Hold the column upright, grasp the lower breakaway end between the thumb and forefinger, and break off. Place the lower end of the column into one of the 2 ml collection tubes provided and lift off the top cap. Save the top cap and white end cap. Except when centrifuging, columns should remain in an upright position.
3. Centrifuge the column for five minutes at 700 x *g*. The column matrix will appear semi-dry upon completion of this step because the equilibration buffer has been removed and the matrix bed compacted.
4. Remove the column and collection tube from the rotor. Discard the collection tube containing the equilibration buffer.
5. Place the column into a second collection tube. Carefully apply the sample to the center of the flat surface of the matrix bed. The sample should not flow along the inner wall of the column to ensure maximum effectiveness. To monitor the loading of the sample onto the gel matrix, add 0.01% (w/v) Bromphenol Blue to the sample. The dye will be removed from the sample with other small molecules during centrifugation.
6. Centrifuge at 700 x *g* for five minutes.
7. Remove the column and collection tube from the rotor. Discard the spin column. The purified sample is in the bottom of the collection tube.

Spin Column Buffer Replacement

(Optional for columns equilibrated in TE or STE)

1. Invert the spin column several times to suspend the matrix uniformly.
2. Hold the column upright, grasp the lower breakaway end between the thumb and forefinger, and break off. Place the lower end of the column into one of the 2 ml microcentrifuge tubes (standard capacity) or one of the sterile 17 x 100 mm collection tubes provided and lift off the top cap. Save the top cap and white end cap. Except when centrifuging, columns should remain in an upright position.
3. Buffer that immediately collects in the 2 ml collection tube should be discarded before proceeding. Reconnect the column to the same collection tube.
4. Centrifuge the column for three minutes at 700 x *g*.

5. The collected buffer should be discarded. Add 1 ml of the desired buffer to a standard column or 3 ml to a large capacity column. After addition of the desired buffer, replace both end caps and invert the column several times to resuspend the gel matrix.
6. Allow the buffer to drain through the column by gravity or centrifuge for three minutes at 700 x *g*.
7. Remove the column from the collection tube, discard the buffer, and replace the column in the collection tube. Apply the new buffer (do not resuspend the matrix) and centrifuge two more times.
8. If the column is to be used immediately, remove the collected buffer and proceed with the purification beginning with Step 3 of the Spin Column Procedure.
9. If the column is to be stored for later use, sodium azide should be added to the buffer to prevent bacterial growth. The equilibration buffer should have a final concentration of 0.1% (w/v) sodium azide. Add 0.7 ml of the buffer containing 0.1% (w/v) sodium azide to a standard capacity column or 2 ml to a large capacity column. Tightly replace the top cap and invert several times to resuspend the gel matrix. Store at 4 °C in an upright position. The columns should be washed twice prior to use, to remove sodium azide. Use 1 ml of equilibration buffer for a standard column or 3 ml of equilibration buffer for a large capacity column.

Note:
The relationship between relative centrifugal force (RCF) and rpm for different centrifuge rotors:

$$rpm = 1000 \sqrt{RCF / (1.12r)}$$

or

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

RCF = relative centrifugal force in units of *g* (x *g)*
r = rotor radius (in mm)
rpm = revolutions per minute

Examples:
i. Swinging bucket rotor: Beckmann Model TJ-6, r =125 mm
   rpm = 2236 results in RCF = 700 x *g*
ii. Fixed-angle rotor: Eppendorf Model 5415C, r = 50 mm
   rpm = 3536 results in RCF = 700 x *g*

References

1. Hagel, L. et al., J. Chromatography, **476**, 329-344 (1989).
2. Porath, J., and Flodin, P., Nature, **183**, 1657-1659 (1959).
3. Sambrook, J. et al. (1989) Molecular Cloning; A Laboratory Manual (2nd ed.) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 6.22-6.34,

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