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SPIN-*Pure*™ G-50 COLUMNS
Instruction Manual

Product:	SPIN-*Pure*™ G-50 COLUMNS (39.5)
Product No:	SCT50-50 TE Buffer
	SCW50-50 DEPC-water
	SCD50-50 Dry
Storage:	Pre-Hydrated columns - store at 4°C
	Dry columns - store at room temperature
Stability:	Pre-Hydrated columns are guaranteed for 6 months from purchase date
	Dry columns are guaranteed for 1 year from purchase date

Principle

SPIN-*Pure*™ G-50 COLUMNS are used for the fast and efficient purification of large molecules (proteins, nucleic acids, complex carbohydrates) from small molecules (nucleotides, labels and buffer salts). The column design is based on the description by Sambrook, *et al.* (1) of gel filtration for the purification of DNA from nick translation reactions. Each unit consists of a special fritted column packed with either prehydrated or dry gel, 2.0 ml wash tube and 1.5 ml sample collection tube.

SPIN-*Pure*™ G-50 COLUMNS are packed with Sephadex® G-50. These columns provide excellent recovery of DNA fragments > 20 bases while removing >98% of salts, and other low-molecular-weight compounds.
The SPIN-*Pure*™ G-50 COLUMNS packed with pre-hydrated gel are ready to use - no equilibration is necessary.
The SPIN-*Pure*™ G-50 COLUMNS packed with dry Sephadex® can be hydrated with reagent-grade water or a suitable buffer for your application prior to use. The SPIN-*Pure*™ G-50 COLUMNS are first spun to remove the interstitial fluid. The sample is then added and the column is spun again to process the sample. The sample is purified by the retention of low-molecular-weight contaminants in the matrix, while the larger molecules of interest are exchanged into the buffer and eluted into the collection tube.

SPIN-*Pure*™ G-50 Columns have been designed specifically for the following uses.

- Removal of unincorporated precursors in random-priming, nick-translation and end labeling reactions
- Removal of dye deoxyterminators in manual or automated sequencing reactions
- Purification of protein conjugates in
  iodination
  cross-linking
  biotinylation
  fluorescence labeling
  haptenation
- Desalting/purification/buffer exchange of proteins prior to electrophoresis

These columns are far superior --- in ease of use, speed, and non-toxicity --- to such common techniques as phenol/chloroform extraction, ethanol precipitation, dialysis and ultrafiltration.
Benefits include:

- RAPID AND EFFICIENT SEPARATIONS
- CHOICE OF PRE-HYDRATED (TE OR DEPC-WATER) OR DRY FOR USE WITH ANY BUFFER
- SAMPLES ARE NOT DILUTED
- 20-50 µl SAMPLE SIZE

Kit Contents:

50 SPIN-*Pure*™ G-50 COLUMNS containing either Pre-hydrated gel (TE or DEPC-Water) or dry media
50 Wash Tubes (2 ml)
50 Sample Collection Tubes (1.5 ml)

Column Hydration (Dry Columns - only)

1. Gently tap the column to insure that the dry gel has settled in the bottom of the spin column.

2. Remove the top column cap and add 0.75 ml of reagent grade water or buffer of choice. Replace the column cap and vortex vigorously for ~10 seconds. Remove air bubbles by sharply tapping the bottom of the column. It is important to hydrate all of the dry gel.

3. Allow a minimum of 30 minutes of room temperature hydration time before using the columns. Reconstituted columns may be stored refrigerated at 4°C for several days. Longer storage can be accomplished in 10 mM sodium azide (NaN_3). Allow refrigerated columns to warm to room temperature before use.

Removal of Interstitial Fluid (All Columns)

1. Remove the top cap of the columns, then the bottom cap. Place the spin column into the 2.0 ml wash tube provided and centrifuge at 770 xg for 2 minutes to remove interstitial fluid. (For example: for Eppendorf Model 5415C, spin at 3000 rpm for 2 minutes.) If you use a fixed-angle microcentrifuge, keep track of the position of the column using the orientation mark molded into the column.

   NOTE: A failure to remove excess interstitial fluid after hydration of the columns can result in ineffective separation. If you see that a column has released less fluid than the others during the first spin, spinning them again briefly will usually remove the excess fluid.

2. Remove the column from the wash tube and place into the 1.5 ml collection tube provided. If there is a drop of liquid on the bottom of the column, blot it dry. Discard the wash tube containing the interstitial fluid. Process the sample within the next few minutes.

Sample Processing (All Columns)

1. Transfer 20 to 50 µl of the sample to the top of the gel.
   NOTE: Carefully dispense the sample DIRECTLY ONTO THE CENTER OF THE GEL BED at the top of the column without disturbing the gel surfaces. DO NOT contact the sides of the column with the reaction mixture or the sample pipet tip, since this can reduce the efficiency of purification.

2. Place the column (which is in the sample collection tube) into the rotor. Maintain proper column orientation. The highest point of the gel media in the column should always point toward the outside of the rotor. Centrifuge at 770 xg for 2 minutes. The purified sample will collect in the bottom of the sample collection tube. Discard the spin column and continue with your procedure. The sample can be stored directly in the sample tube.

EXPECTED RECOVERIES

Oligo Recovery		Protein Recovery		
Size of DNA	% Recovery	Protein	MW(kDa)	% of Recovery
NTP	<0.01	IgG	150	55-87
11-mer	<2	BSA	66	14-29
15-mer	39	Protein A	42	13-18
20-mer	72			
24-mer	72			
28-mer	81			

Centrifuge Notes

Maximum yield and efficiency are obtained with horizontal or swinging-bucket rotors. However, fixed-angle-rotor microcentrifuges will provide acceptable performance and save time.

If using a variable speed microcentrifuge, DO NOT use the pulse button, which overrides the speed setting and takes the rotor to maximum g-force. If you are not sure of the g-force generated by your centrifuge at specific speeds, calculate the correct speed by using the following formula:

$$rpm = \sqrt{\frac{RCF}{(1.12 \times 10^{-5})\, r}}$$

rpm = revolutions per minute;
RCF = Relative Centrifugal Force
r = radius (cm) measured from center of spindle to bottom of rotor bucket.

Reference

1. Sambrook, J., Fritsch, E.F., and Maniatis, T., *Molecular Cloning: A Laboratory Manual,* Cold Spring Harb Laboratory, 1989.

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