

CentriSpin™-20 Columns Protocol:

CentriSpin-20 columns have been designed specifically for the following uses:

- Removal of free and labelled dNTPs from DNA/RNA as in:
 - nick translation
 - end-labeling reactions
 - PCR-reactions
- Primer removal
- Removal of hexamers and octamers from primer walking and random primer labelling.
- Desalting, removal of traces of phenol or exchange of buffer salts, as in multiple restriction digestions.
- Desalting/purification/buffer exchange of peptides or proteins > 25 kDa.

1.0 Column hydration

- 1.1 Gently tap the column to insure that the dry gel has settled in the bottom of the spin column.
- 1.2 Remove the top column cap and reconstitute the column by **adding 0.65 ml** of reagent grade water or buffer. Replace the column cap and vortex vigorously for about 5 seconds. Remove air bubbles by sharply tapping the bottom of the column. It is important to hydrate **all** of the dry gel.
- 1.3 Allow at least 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 (NaN₃).

Allow refrigerated columns to warm up to room temperature before use!

2.0 Removal of interstitial fluid

- 2.1 After the gel has hydrated and is free of bubbles, first remove the top column cap, and then remove the column end stopper from the bottom.
- 2.2 Spin column and wash tube in a variable speed centrifuge at 750 x g for 2 minutes to remove interstitial fluid. For 7.5 cm rotors (Standard table top centrifuges) the correct speed is 3,000 rpm.

If you use a microcentrifuge, it is important to keep track of the position of the column using the orientation mark molded into the column.

- 2.5 If there is a drop at the end of the column, blot it dry. Discard the wash tube and the interstitial fluid. Do not allow the gel material to dry excessively.

Process sample within the next few minutes!

3.0 Sample processing

- 3.1 Hold the column up to the light. Transfer 20 to 50 µl of sample to the top of the gel. Carefully dispense the sample **DIRECTLY ONTO THE CENTER OF THE GEL BED** at the top of the column, without disturbing the gel surface. **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 and possibly ruin the analysis due to excess dye.
- 3.2 Place the column into the **SAMPLE COLLECTION TUBE** (1.5 ml) and place both into the rotor. **Maintain proper column orientation (molded mark)**. The highest point of the gel media in the column should always point toward the outside of the rotor. Spin the column and collection tube at **750 x g for 2 minutes**. The purified sample will collect in the bottom of the sample collection tube. Discard the spin column and proceed with your procedure.

Experimental results

Size of DNA	% recovery
NTP	0
11-mer	< 2
15-mer	39
20-mer	72
24-mer	71
28-mer	81

