CentriPure Columns

Removal of Dye Terminators prior to sequencing:

CentriPure columns are used for quick and efficient desalting, buffer exchange, dye terminator removal and removal of labels from labelling reactions. The columns must be hydrated with either water or a buffer of choice before use.

CentriPure columns are designed for research use only.

Product specifications

Gel Matrix	Zetadex-50SF
Gel bed volume	0.5mL
Buffer conductifity	Less than 300µS/cm
Sample volume	Up to 100µL
Optimal sample size	20µL to 50µL
DNase activity	Not detected
Optimal centrifuge conditions	1000 x g for 2 minutes
Removal of fluorescein (50µL 1mM 5/6- carboxyfluorescein in 0.1M NaHCO3)	>99.999%
Removal of salt (50µL 0.8M NaCl)	>99.9% >99.999% (with extra wash step)
Recovery	Will vary depending on sample and buffer conditions.
Size of eluted oligos	>20 bases
Size of eluted protein	>25 kDa



Protocol:

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 pipette 650µL of deionized water (or buffer) to the dry gel bed.
- 1.3 Replace the top cap and briefly vortex to thoroughly suspend and hydrate the gel.
- 1.4 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 (NaN3)).

Allow refrigerated columns to warm up to room temperature before continuing this procedure!

2.0 Removal of interstitial fluid

- 2.1 Invert the column and vortex or tap sharply to remove air bubbles. Allow column to stand upright until the gel fully settles.
- 2.2 Remove the top cap and then remove bottom cap.
- 2.3 Place the column into a wash tube.
- 2.4 Centrifuge the column and wash tube at 1000 x g for 2 minutes.
 Note: It is important to keep track of the position of the column using the orientation mark molded into the column!
- 2.5 Discard the wash tube and eluted void volume. Blot the bottom of the column dry!
- 2.6 For maximum desalting efficiency, add 400μL destilled water to the column and repeat steps 2.1 to 2.5. Do not allow the gel material to dry excessively.

Process sample within the next few minutes!

3.0 Sample processing

- 3.1 Carefully apply sample directly to the center of the gel bed (see Fig.). Carefully dispense the sample at the top of the column, without disturbing the gel surface.
 DO NOT contact the sides of the column with the sample or the sample pipette tip, since this can reduce the efficiency of purification and possibly ruin the analysis.
- 3.2 Place column into a SAMPLE COLLECTION TUBE (1.5mL) 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.
- 3.3 Centrifuge at 1000 x g for 2 minutes. The purified sample will collect in the bottom of the sample collection tube.



