ReadiUse™ Disposable PD-10 Desalting Column

Catalog number: 60504, 60505
Unit size: 5 Columns, 10 Columns

Component	Storage	Amount (Cat No. 60504)	Amount (Cat No. 60505)
ReadiUse™ Disposable PD-10 Desalting Column	Refrigerated (2-8 °C)		

OVERVIEW

ReadiUse™ Disposable PD-10 Desalting Columns contain Sephadex G-25 resin for rapid buffer exchange, desalting, and removal of small contaminants (e.g., salts, dyes, radioactive labels) from samples using gravity flow or centrifugation. It is an inexpensive and convenient alternative to lengthy and tedious dialysis procedures. The desalting and buffer exchange is run by gravity flow (1.0 to 2.5 mL samples) or centrifugation (1.75 to 2.5 mL samples). The salt removal rate is typically >98% salt with gravity and >90% with centrifugation. The sample recovery is typically in the range of 70% to >95%.

SAMPLE EXPERIMENTAL PROTOCOL

Spin Protocol

1. PD-10 Desalting column preparation
   - Cut off the tip at the notch and place the column on a rack. Remove the cap to allow the excess packing buffer to drain by gravity until reaching the top of the gel bed. If the column does not begin to flow, push the cap back into the column and remove it again to start the flow. Discard the flow-through.
   - Put the reservoir to the top of the column as shown in **Figure 1**.
2. Column equilibration
   - Fill the reservoir with the equilibrium buffer of your choice (for example, PBS). Let the buffer drain out by gravity.
   - Repeat 2 times and discard the flow-through.
3. Sample application
   - Remove the buffer reservoir and place the column in a 50 mL centrifuge tube with a spin adapter (**Figure 2**).
   - Centrifuge for 5 min in a swinging bucket centrifuge at 1,000x g to remove the reaction buffer. Discard the flow-through.
   - Place the column in **a clean** 50 mL centrifuge tube with a spin adapter.
   - Carefully apply the sample (1000 µL) directly to the top center of the column. Sample volume may need to be carefully adjusted to reach the best performance.
4. Elution
   - After loading the sample, add 100 µL equilibrium buffer slowly in the middle of the packed bed and centrifuge the column for 10 min at 1,000 x g.
   - Collect the eluate.

Note. The ReadiUse™ PD-10 Column can fit into a 50 mL centrifuge tube with a PD-10 spin adapter for sample collection during centrifugation.

Swinging bucket centrifuges capable of generating a minimum force of 1,000g are suitable for ReadiUse™ PD-10 column use. The gravitational force created at a particular revolution speed is a function of the radius of the centrifuge rotor. Consult the swinging bucket centrifuge instruction manual for the information about conversion from revolutions per minute (RPM) to centrifugal or g-force. Alternatively, use the equation below to calculate the speed in RPM required to reach the gravitational force of 1,000 x g.

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

- RCF = the relative centrifugal force
- RPM = the speed of the rotor
- r = the radius in centimeters measured from the center of the rotor to the middle of the PD-10 column

Gravity Protocol

The liquid passes through the column by gravity force. There is a slightly higher recovery and desalting capacity using the gravity protocol than the spin protocol, but the applied sample is diluted.

If the sample has color, you can choose the Gravity Protocol. If the sample is clear, the Spin Protocol is recommended.

1. PD-10 Desalting column preparation
   - Cut off the tip at the notch and place the column on a rack. Remove the cap to allow the excess packing buffer to drain by gravity until reaching the top of the gel bed. If the column does not begin to flow, push the cap back into the column and remove it again to start the flow. Discard the flow-through.
2. Column equilibration
   - Fill up the column with the equilibration buffer and allow the equilibration buffer to enter the packed bed completely.
   - Repeat several times. ~25 mL equilibration buffer should be used to equilibrate the column.
   - Discard the flow-through.
3. Sample application
   - Add 1.0 mL of sample to the column.
   - Wait until it enters the packed bed completely.
4. Elution (volumes may need to be adjusted based on different samples)
   - Add 1.0 mL equilibration buffer at a time. After it enters the packed bed completely, add another 1.0mL.
   - Discard the first 2.0 mL flow-through solution and start to collect elution from 3.0 mL.

- Collect ~ 2.0-3.0 mL eluate. (Note: 0.5~1 mL/fraction)

EXAMPLE DATA ANALYSIS AND FIGURES

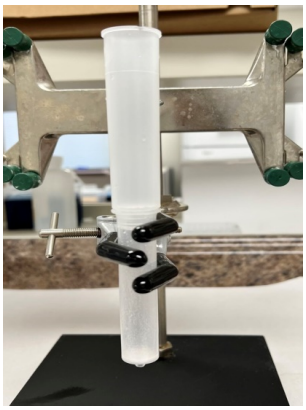

Figure 1. Column equilibration.

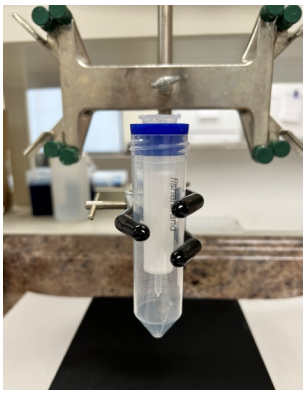

Figure 2. Sample Application.

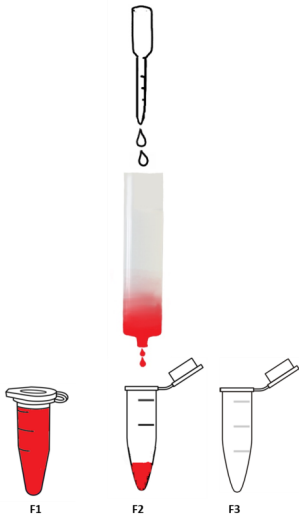

Figure 3. Gravity Purification with ReadiUse™ PD-10 column.

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