

ReadiUse™ Bio-Gel P-6 spin column

Catalog number: 60500

Unit size: 5

| Component | Storage | Amount |
|-------------------------|------------------|----------------|
| Bio-Gel P-6 spin column | Room temperature | 5 spin columns |

OVERVIEW

ReadiUse™ Spin chromatography columns are packed with polyacrylamide P-6-DG matrices for buffer exchange and desalting applications. When applying a material to the spin column, the substances smaller than the column's exclusion limit (MW~6000) will be retained in the column and large molecules like proteins (MW is above 15,000) will be eluted with a buffer. The sample volume ranges from 30 uL to 100 uL. They are compatible with swinging bucket centrifuge with 2.0 ml microcentrifuge tubes or 12 x 75 test tubes.

SAMPLE EXPERIMENTAL PROTOCOL

1. Invert the Spin Column several times to suspend the settled gel and remove any bubbles.
2. Snap off the tip and place the column in a microcentrifuge tube (2 mL). Remove the cap to allow the excess packing buffer to drain by gravity until reaching the top of the gel bed. If column does not begin to flow, push cap back into column and remove it again to start the flow. Discard the drained buffer, and then place the column back into the Tube.
3. Centrifuge for 1 min in a swinging bucket centrifuge at 1,000 x g to remove the packing buffer. Discard the buffer.
4. Apply 1 mL equilibrium buffer of your choice to the column, let the buffer drain out by gravity, or centrifuge the column for 1 min to remove the buffer. Discard the buffer from the collection tube. Repeat this process for 3-4 times.
5. Centrifuge for 2 minutes in a swinging bucket centrifuge at 1,000 x g to remove the reaction buffer. Discard the buffer.
6. Place the column in a clean 2.0 ml microcentrifuge tube or 12 x 75 mm test tube. Carefully apply the sample (20–100 µl) directly to the top center of the column. Sample volume may need be carefully adjusted to reach the best performance.
7. After loading sample, centrifuge the column for 5 min at 1,000 x g.
8. Following centrifugation, the purified sample is now in the equilibrium buffer and molecules smaller than the column's exclusion limit (~6000) will be retained.
9. Collect the elution, and properly dispose the used column.

Note Spin Column can fit into 2 mL microcentrifuge tubes or 12 x 75 mm test tubes for sample collection during centrifugation. Use the 2 mL microtubes provided with the columns for the initial column equilibration step.

Note Swinging bucket centrifuges capable of generating a minimum force of 1,000 x g are suitable for Bio-Spin column use. The gravitational force created at a particular revolution speed is a function of the radius of the microcentrifuge 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 shown below to calculate the speed in RPM required reaching 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 Bio-Spin column

EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. Prepacked ReadiUse™ Bio-Gel P6 Spin Column.

The column was packed with P-6 DG in PBS buffer for sample volume 50~100 uL. The MW limit is ~6000 and large molecules like proteins (MW > 15,000) will be eluted with a buffer.

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