PRO•SPIN™

Spin Columns

for Purification
of Dye or Hapten Labeled IgG

Pro•Spin™ spin columns are designed for the removal of unbound fluorescent dyes, haptens or chromophores from labeled IgG antibody. They are useful for buffer exchange and salt removal. (Please refer to the "General Tips" section for assistance in the purification of other types of proteins.)

Each purification unit consists of (1) a special fritted microfuge tube containing gel filtration media, (2) a wash tube and (3) a sample collection tube.

The column media is hydrated with buffer (See note on hydration buffer in Step 2). It is spun under centrifugal force to remove excess fluid using a microcentrifuge. The IgG sample is loaded onto the column, and spun again, to recover purified labeled IgG.

Pro•Spin™ spin columns offer several benefits that include:

Versatility
- Removal of dyes, haptens or salts

Efficiency
- > 90% recovery of labeled IgG

Simplicity
- Requires only a table top centrifuge

Speed
- Two 2-minute centrifugation steps

Reliability
- Consistent results

Convenience
- Room temperature storage

Stability
- Extended shelf life

PRINCETON SEPARATIONS

Protocol for 1 mg Labeled IgG

(1) The sample volume capacity of each spin column is ≤100 uL. If the sample volume is greater than 100 uL, it can be divided into aliquots and applied to separate spin columns.

Column Hydration

(2) Gently tap the columns to insure that the dry gel has settled to the bottom of the column. Remove the top column caps and reconstitute all columns by adding 650 uL of buffer

Note on Hydration Buffer:

Hydrate the column with PBS buffer. Buffers other than PBS can be used. However, these may affect non-specific binding of the IgG to the column via ionic or hydrophobic interactions. These interactions may affect recovery of the labeled IgG from the column.

(3) Replace the column cap and vortex vigorously for about 5 seconds. Remove air bubbles by sharply tapping the bottom of the columns. Allow at least 30 minutes of room temperature hydration time before using the columns.

Note on Column Hydration:

It is important to hydrate <u>all</u> of the dry gel.

(4) After 30 minutes of hydration, remove the top column caps and then remove the column end stoppers from the bottom.

Note on Column Storage:

Hydrated columns may be stored at 4°C for several days. Longer storage can be accomplished in 10 mM sodium azide. Allow refrigerated columns to warm to room temperature before use.

(5) Spin the columns in their wash tubes in a variable speed centrifuge at 750 x g for two minutes to remove excess fluid.

Note on Fixed Angle Microcentrifuge:

If you use a fixed-angle microcentrifuge, keep track of the position of the columns using the orientation mark molded into the columns.

Calculating Correct Centrifugal Speed

Maximum yield and efficiency are obtained with a horizontal or swinging-bucket rotor. However, fixed-angle-rotor microcentrifuges provide acceptable performance and save time. On a variable speed microcentrifuge, <u>do not use the pulse button</u>, 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:

$$RPM = \sqrt{\left[RCF \div (1.119 \times 10^{5} \times r)\right]}$$

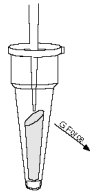
Where:
- *RPM is revolutions per minute*
- *RCF is relative centrifugal force*
- *r is the radius in centimeters from the center of the spindle to the bottom of the bucket.*

(6) Blot excess drops from the bottom of the column. Discard the wash tubes and the excess fluid. Do not allow the gel material to dry excessively. Process the samples within the next few minutes.

Purification of Antibody Conjugate

(7) Hold the columns up to the light. Transfer ≤100 uL of the sample (Refer to Step 1) to the top of the gel of each column.

Note on Loading Columns



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 touch the sides of the columns with the reaction mixture or the sample pipet tip since this can reduce the purification efficiency.

(8) Place each column into a collection tube and place both together into the rotor. Maintain proper column orientation.

Note on Column Orientation

The highest point of the gel media in the column should always point towards the outside of the rotor.

(9) Spin the columns and collection tubes at 750 x g for 2 minutes. The purified IgG conjugate will collect in the bottom of the collection tubes.

(10) Discard the spin column(s).

(11) Store the purified IgG under appropriate conditions.

Labeled IgG Recovery

Greater than 90% recovery of both heavy and light chains of fluorescein labeled rabbit IgG from the spin column using PBS hydration buffer as determined by UV-VIS A280 absorption.

Materials Provided

- 50 Pro•Spin Columns
- 50 Wash Tubes
- 50 Sample Collection Tubes

Additional Materials Recommended

- Microcentrifuge
- Pipet
- Pipet tips
- Microtube rack
- Vortex mixer

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PRINCETON SEPARATIONS

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Ordering Information

Product	Catalog Number
Pro•Spin	CS-800

General Tips for Labeled Protein Purification

Dyes, haptens, chromophores and salts can be removed from proteins other than IgG using Pro•Spin spin columns. However, recovery of the protein may be reduced due to specific or non-specific interactions with the column matrix. Below is a list of common modes of interaction and other problems together with suggestions for their minimization.

Problem	Possible Solution
Hydrophobic interaction with column matrix	Add non-ionic detergent, decrease ionic strength or increase pH of hydration buffer
Ionic interaction with column matrix	Increase ionic strength of hydration buffer
Sample precipitation	Increase ionic strength, remove salt from hydration buffer or dilute sample.
Protease degradation of sample	Add suitable protease inhibitor to hydration buffer.

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