INSTRUCTIONS

Immobilized Jacalin

3747 N. Meridian Road
P.O. Box 117
Rockford, IL 61105

20395

0735

Product Description

Number	Description
20395	**Immobilized Jacalin,** *5 ml gel*
	See product label for lot-specific binding capacity.
	Loading: Approximately 4.5 mg Jacalin per ml of gel.
	Store at 4°C. Do not freeze.

Introduction

Jacalin is an α-D-galactose binding lectin extracted from jack-fruit seeds (*Artocarpus integrifolia*).[1] This lectin is a glycoprotein of approximately 40,000 MW composed of four identical subunits. Jacalin immobilized on supports such as agarose has been useful for the purification of human serum or secretory IgA1.[2-12] IgA can be separated from human IgG and IgM in human serum or colostrum.[2] IgD is reported to bind to jacalin.[4] This support is also useful for removing contaminating IgA from IgG samples. Immobilized jacalin is preferable to protein A because protein A will bind some IgA along with IgG and is not effective for IgA removal.[3]

Materials

A. Immobilized Jacalin, 5 ml
B. Human Serum
C. Binding Buffer: Phosphate buffered saline (PBS), 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.4
D. Elution Buffer: 0.1 M melibiose or 0.1 M α-D-galactose in PBS
E. Column

Packing a Column

1. Allow the gel slurry and deionized water to come to room temperature.
2. Place the bottom cap on the end of a column.
3. Place the column in a test tube and add 2.75 ml of degassed water to a 2.0 ml column, 6.0 ml to the 5.0 ml column or 12.0 ml to the 10.0 ml column. Tap the end of the column sharply to dislodge any air bubbles. A 0.035% solution of detergent such as Surfact-Amps™ X-100 (Product No. 28314) can be substituted for degassed water to make it easier to remove entrapped air.
4. Float one of the porous Polyethylene discs on top of the liquid within the column.
5. Use the reverse end of a serum separator or pipette tip to push the disc to the bottom of the column. As the disc passes to the bottom, the air will be purged out through the disc.
6. Empty the column of water and add gel slurry to the column.
7. Allow 30 minutes for the gel to settle in the column.

8. Place one of the discs on top of the liquid within the column and depress it to just above the settled gel level. Leave approximately 1-2 mm of space between the top of the gel bed and the bottom of the top disc.
9. Wash the inside top part of the column to clean out any gel that may have remained along the sides during packaging. The column is now ready for use. It may be stored in 0.02% sodium azide at 4°C.

Method

1. Equilibrate the column with at least 5 column volumes of binding buffer.
2. Mix human serum 1:1 with binding buffer, centrifuge if necessary and add to column.
3. Wash the column with 5 column volumes of binding buffer or until baseline is reached using absorbance at 280 nm to measure effluent.
4. Elute the bound IgA1 using 2 ml aliquots of elution buffer until baseline absorbance is reached. Collect the eluate from the aliquots in separate test tubes and monitor absorbance at 280 nm to determine baseline.
5. Buffer exchange the sample into binding buffer using a gel filtration/desalting column to remove the melibiose or galactose.
6. Regenerate the column by washing with 20 column volumes of PBS.

References

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