INSTRUCTIONS

UltraLink™ Immobilized Jacalin

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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 secratory 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 preferrable to protein A because protein A will bind some IgA along with IgG and is not effective for IgA removal.[3]

UltraLink™ Immobilized Jacalin is jacalin on the azlactone-activated support 3M Emphaze™ Biosupport Medium AB 1. This activated matrix has been coupled to a variety of ligands. 3M Emphaze™ Biosupport Medium AB 1 is a hydrophilic, high capacity, highly crosslinked, copolymeric, porous support. The reactive groups couple to amines yielding a stable bond and minimal ligand leakage. UltraLink™ supports are more sturdy than agarose allowing for their use in FPLC and large-scale applications.

Characteristics of 3M Emphaze™ Biosupport Medium AB 1

Capacity:	See label for specific capacity
pH stability of matrix:	1-13
Particle Size (average)	50-80 microns (typically 60 µm)
Exclusion limit (proteins):	>2,000,000 daltons
Surface area (average):	>250 m^2/g of beads
Pore volume (average):	>1.2 ml/g of beads (>60% of bead volume)
Pore size:	1000 Å
Maximum pressure:	100 psi (6.9 bar)*
Maximum linear velocity:	3000 cm/hour

***NOTE:** The indicated maximum pressure of 100 p.s.i. refers to the maximum pressure drop across a column the support can withstand. It does not necessarily refer to the indicated system pressure shown on a liquid chromatography apparatus, since the system may not actually be measuring the pressure drop across the column.

Product Description

NUMBER	DESCRIPTION
53145	**UltraLink™ Immobilized Jacalin,** *5 ml gel* See product label for lot-specific binding capacity. Loading: Approximately 2-3 mg Jacalin per ml of gel. Store at 4°C. Do not freeze.

Sample Protocol for Medium Pressure Chromatography

UltraLink™ supports are extremely valuable for use in medium pressure chromatography applications. When packed into a 3 mm (diameter) x 14 cm (height) glass column, UltraLink™ supports have been run up to approximately 400 p.s.i. (system pressure) with no visual compression of the gel or adverse effects on the chromatography. Typically these columns were run at linear velocities of 85-3000 cm/hour with excellent separation characteristics. Purification time can be reduced from 8-12 hours to several minutes when an FPLC procedure is used instead of a gravity flow method.

Medium Pressure Chromatographic Applications

The 3M Emphaze™ Biosupport Medium can be used in Fast Perfomance Liquid Chomatography (FPLC) applications. With MPC (medium pressure chromatographic) applications the critical factor for success is limiting the pressure drop across the column that the support experiences. Due to variations with the instrument, the indicated guage pressure of an MPC apparatus may not actually measure the pressure drop across the column.

A more universal criteria for MPC applications, therefore, would be to measure the linear velocity of the buffers through the column, a pressure-independent measurement. The linear velocity is defined as the velocity of the buffer front passing through the gel bed. Linear velocity is usually expressed in cm/hour, and in the case of the 3M Emphaze™ Biosupport Medium, the maximum linear velocity that the support can tolerate in a 1 cm diameter by 10 cm high bed is 3000 cm/hr.

The linear flow rate through a cylindrical column can be calculated if the inside diameter of the column is known and the column effluent is collected and measured for a given time to determine the flow rate. The calculations for determining linear velocity are shown on the following page:

Radius measured in cm = r

Column cross-sectional area in square centimeters = πr^2

1 ml = 1 cubic centimeter

Measured flow rate (ml/minute) = Column effluent collected in ml/minute

$$\text{Linear velocity per minute} = \frac{\text{(measured flow rate in ml/minute or cm}^3\text{/minute)}}{\text{cross-sectional area in cm}^2}$$

Linear velocity per hour = (linear velocity per minute)(60 minutes/hour)

$$\frac{\text{Measured flow rate per hour}}{\text{Column cross-sectional area}} = \frac{\text{(cm3/minute)(60 minute/hour)}}{\pi r^2} = \text{Linear velocity expressed as cm/hour}$$

Materials

A. UltraLink™ Immobilized Jacalin, 5 ml

B. Human serum

C. Binding buffer: Phosphate buffered saline (PBS), 0.1 *M* sodium phospate, 150 mM NaCl, pH 7.4

D. Elution buffer: 0.1 *M* melibiose or 0.1 M α-D-galactose in PBS

E. Column

Method

1. Gravity pack under flow UltraLink™ Jacalin into a medium pressure chromatography column.
2. Store the column in 0.02% sodium azide if column will not be used immediately.
3. Equilibrate the column with at least 5 column volumes of binding buffer.
4. Add human serum to binding buffer in equal volumes, centrifuge if necessary and add or inject supernatent onto column.
5. Wash the column with an additional 8 column volumes of binding buffer or until baseline is reached using absorbance at 280 nm to measure effluent.

6. 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.
7. Buffer exchange the sample into binding buffer using a gel filtration/desalting column to remove the melibiose or galactose.
8. Regenerate the column by washing with 20 column volumes of PBS.

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