# S sterogene bioseparations

#### **Jacalin Superflow 4**

#### Instructions for Use

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Jacalin Superflow 4 is an affinity resin for the purification of  $\alpha$ -D galactose-containing proteins. Purified Jacalin is immobilized covalently to our highly crosslinked 4% agarose beads. The coupled resin is highly stable and shows no leaching with commonly used buffers and at pH range from 3-12. Jacalin binds strongly and specifically to IgAs and thus Jacalin Superflow 4 resin can be used to efficiently purify IgAs from various biological samples (serum and colostrum).

Jacalin is a plant lectin isolated from Jackfruit (*Artocarpus integrifolia*) seeds using D-galactose affinity resin. It is a tetrameric lectin (Molecular weight of 66.0 kDa) composed of dimers of two different subunits (molecular weight of 16.4 and 17.1 kDa, respectively) held together by non-covalent linkages. Jacalin can be used efficiently in localization studies and purification of glycoproteins specifically containing *O*-glycosidically linked oligosaccharides.

Jacalin Superflow 4 is supplied as 50% slurry. Upon receiving, store the resin at 2-8 °C. Do not freeze.

## **Key features:**

Matrix support: 4% crosslinked agarose beads

Immobilized ligand: Jacalin

Ligand density: 5 mg lectin/ml of agarose beads

Binding capacity: 3-5 mg of human IgAs/ml of resin

Storage buffer: 10 mM phosphate buffer, 150 mM NaCl, pH 7.5, with 0.05% sodium azide

Elution buffer: 0.1-0.3 M  $\alpha$ -D-galactose or 0.1 -0.3 M melibiose in PBS

#### **Protocol:**

### Purification of IgAs from serum

Jacalin Superflow 4 resin can be packed in a disposable column (1-5 ml) or can in a spin column depending on the scale of purification. Dilute the serum 1:1 in equilibration buffer (10 mM phosphate buffer, 150 mM NaCl, pH 7.5 PBS)

- 1. Remove the resin from the fridge and equilibrate it at room temperature.
- 2. Pack the required amount of slurry to the column.
- 3. Equilibrate the packed column with 5 column volumes (CV) of PBS.
- 4. Apply the diluted serum on the column with slow flow rate.
- 5. Wash the column with 5-10 CV of PBS at high flow rate followed by additional 5 CV wash with high salt (PBS containing 500 mM NaCl, pH 7.5) until no protein is detected in the flowthrough, based on OD at 280 nm.
- 6. Elute the bound IgAs with 0.1-0.3 M solution of 0.1-0.3 M  $\alpha$ -D-galactose or melibiose in PBS at slow flow rate and in multiple small fractions.
- 7. Take the OD at 280 nm to determine the concentrated fractions, run the SDS-PAGE and pool the fractions, dialyze in required buffer, concentrate, and store at required temperature.
- 8. The Jacalin Superflow 4 resin can be regenerated by washing the column with more than 20 CV of PBS. The clean column is then ready for another round of purification or can be stored at 4 °C in storage buffer.

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