

Melibiose-Sepharose[®] Beads

09/20

CATALOG # M1323-1	1 ml
M1323-5	5 ml
M1323-25	25 ml

INTRODUCTION:

Melibiose is a reducing disaccharide formed between galactose and glucose by an α -1,6 linkage. It differs from lactose where the galactose and glucose are bonded through a β -1, 4 linkage. Immobilized melibiose is widely used in the affinity purification of various melibiose binding proteins, such as lectins or galactose binding proteins. **BioVision's high-quality Melibiose-Sepharose[®] Beads** exhibit specific, high-yield purification of lectins from various sources.

PREPARATION:

Melibiose-Sepharose Beads are prepared by covalently coupling melibiose to 6% cross-linked Sepharose beads. The coupling technique is optimized to provide a higher binding capacity for lectins, such as Jacalin and minimal leaching of melibiose. The binding capacity of Melibiose-Sepharose is ≥ 10 mg Jacalin per ml of wet beads.

APPLICATION:

Purification of lectins that have binding affinity for melibiose or one of its monomers such as galactose.

CONTENTS: Supplied as 50% slurry in 20% Ethanol/H₂O.

STORAGE: Store at 4°C. Do not freeze. Stable, as supplied for at least one year.

BINDING CAPACITY: ≥ 10 mg Jacalin per ml Melibiose-Sepharose.

***FLOW RATE:** 2.0 ml/min

**Test condition: Calculations are based on the time required to pass 18 ml of water based liquid phase through 2 ml settled beads (column diameter of 1.5 cm).*

USAGE: Reusable for up to 10 times without any significant loss of binding capacity.

PROTOCOL:

1. Carefully pack the column avoiding air bubbles.
2. Equilibrate the column with 5X resin volume of Binding Buffer and allow it to drain through the column. **Note:** Do not let the resin bed dry.
3. Dilute the sample or *extract with Binding Buffer (1:1 ratio) and mix well. ***Note:** For lectin extraction from lentils, soak 0.5 g lentil powder in 10 ml of PBS. Incubate at 4°C overnight. Homogenize and sonicate the solution. Centrifuge at 10,000 rpm for 30 min and 4°C and collect the lectin extract supernatant.
4. Make sure there are no bubbles in the diluted sample.
5. Apply the diluted sample onto the column. **Note:** Do not let the resin bed dry.
6. Collect the flow-through.
7. Reapply the flow-through to the column & collect the sample. Repeat 2 times.
8. Wash the column 4 - 5 times with 5X column volume of Binding Buffer containing 0.5 M NaCl.
9. Wash the column 4 - 5 times with 5X column volume Binding Buffer.
10. Elute the proteins with Elution Buffer ~3-5X resin bed volume.
11. Collect fractions using a micro centrifuge tube.
12. Assay the protein concentration by measuring the absorbance at 280 nm and combine the eluted fractions with the highest absorbance.
13. Remove melibiose or galactose from eluate by gel filtration/desalting column/dialysis.
14. To regenerate and/or store the column:
 - a. Wash the column with 5 column volumes of Elution Buffer.
 - b. Wash the column with 5 column volumes of distilled water.
 - c. Store the column in 20% Ethanol/H₂O at 4°C.

BUFFERS:

Binding Buffer: 0.1 M Sodium Phosphate, pH 7.0

Elution Buffer: 0.1 M Melibiose or 0.1 M α -D-galactose in 0.1 M Sodium Phosphate, pH 7.0

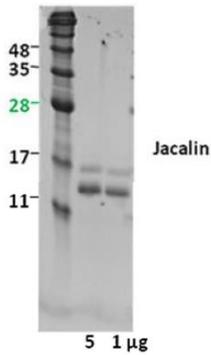


Figure: Melibiose-Sepharose is used in the purification of Jacalin from jack fruit seed extract. Lane 1: Marker; Lane 2: Jacalin protein purified using BioVision's Melibiose-Sepharose Beads.

Jacalin loaded on a
17% SDS-PAGE gel

RELATED PRODUCTS:

- Recombinant Protein A & Agarose, Sepharose & Magnetic Beads (# 6500, # 6500B, # 6526, # 6501, # 6507)
- Recombinant Protein G & Agarose, Sepharose & Magnetic Beads (# 6510, # 6513, # 6511, # 6517)
- Recombinant Protein L & Sepharose & Magnetic Beads (# 6530, # 6531, # 6537)
- Recombinant Protein A/G & Sepharose & Magnetic Beads (# 6502, # 6503, # 6527)
- Recombinant Protein A/G/L & Sepharose & Magnetic Beads (# 6540, # 6541, # 6547)
- Protein A Polyclonal Antibody (# 5500)
- Protein G Polyclonal Antibody (# 5510)
- Protein L Polyclonal Antibody (# 5530)
- 5" Polypropylene Disposable Gravity Column (Cat# M1314)

FOR RESEARCH USE ONLY! Not to be used on humans.