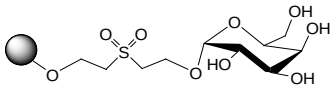
INSTRUCTIONS

Immobilized D-Galactose Gel

20372

1841.1

Number	Description
20372	**Immobilized D-Galactose Gel,** 5mL, supplied as 50% slurry in 0.02% sodium azide Support: Crosslinked 6% beaded agarose Binding Capacity: ≥ 20mg of jacalin/mL of settled gel 

Storage: Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

Thermo Scientific™ Immobilized D-Galactose Gel enables purification of lectins, toxins, and other galactose-, *N*-acetylgalactosamine- or carbohydrate-binding proteins. This purification method may be more mild or specific than other methods, allowing protein recovery in a manner that preserves activity for downstream applications.

Additional Materials Required

- Binding Buffer: For example, 0.1M sodium phosphate, 0.15M sodium chloride, pH 7.2 (Product No. 28372)
- Sample containing galactose-binding proteins dissolved with or dialyzed against Binding Buffer
- Elution Buffer: 0.1M D-galactose in Binding Buffer
- Empty gravity-flow chromatograph column such as the Disposable Polypropylene Columns (2-10mL bed volume; Product No. 29922) or the Column Trial Pack (Product No. 29925) that contains two each of three column sizes.

Example Procedure for Protein Purification using Immobilized D-Galactose Gel

The following is a generalized procedure derived from literature examples. Specific applications may require optimization.

A. Prepare the Affinity Column

1. Insert a bottom frit into an empty chromatograph column and install the bottom cap.
2. Add the desired amount of Immobilized D-Galactose Gel slurry to the column and allow gel to settle.
3. Insert the top frit into the column and push it down to within 1mm of the gel bed.
4. Suspend the column over a collection vessel. Remove the bottom cap and allow the liquid to drain.
5. Equilibrate the column by adding 10 gel-bed volumes of Binding Buffer and allowing it to flow-through.

B. Purification of Galactose-binding Protein

1. Apply the prepared sample to the column and allow column to drain. Continue to add sample until the desired amount has passed through the column.
2. Wash the column with 20-30 gel-bed volumes of Binding Buffer or until the absorbance at 280nm of the flow-through approaches baseline. The column may require extensive washing to remove all nonbound proteins.
3. Recover the bound protein by adding 5-10 gel-bed volumes of Elution Buffer to the column and collect 0.5 or 1mL fractions. Depending on the protein, more Elution Buffer may be required.
4. Measure the absorbance at 280nm of each fraction to determine which fractions contain the eluted protein.

5. Pool the fractions containing protein and dialyze extensively against Binding Buffer (or other suitable buffer) to remove free D-galactose.
6. For storage, wash column with five gel-bed volumes of water containing 0.02% sodium azide. When 0.5-1mL of solution remains above the top frit, replace the bottom cap followed by the top cap. Store column upright at 4°C.

Troubleshooting

Problem	Possible Cause	Solution
Protein binding does not occur	Protein sample does not bind galactose	Determine if proteins are known to bind galactose and determine the optimal binding conditions
	Protein is inactive	Extract the proteins in a manner which preserves activity
Proteins do not elute	Protein did not bind	Verify the protein did bind the column
	Elutions conditions are not appropriate	Use a stronger elution such as 0.1M glycine-HCl, pH 2.3

Related Thermo Scientific Products

28372 **BupH™ Phosphate Buffered Saline Packs,** 40 packs

69550 **Slide-A-Lyzer® MINI Dialysis Units, 3.5K MWCO,** 50/pkg

Additional Information

Please visit the website for additional information on this product including the following:

- Tech Tip #43: Protein stability and storage
- Tech Tip #20: Dialysis: an overview
- Tech Tip #6: Extinction coefficients guide

General References

Alperin, D. M., *et al*. (1992). Isolation, by affinity chromatography and gel filtration on 8 M-urea, of an active subunit from the anti-(blood-group A+N)-specific lectin of *Moluccella laevis*. *Biochem J* **285(Pt 1):**1-4.

Hermanson, G. T., *et al.* (1992). *Immobilized Affinity Ligand Techniques*. Academic Press. 321-28.

Tulasi, R.B. and Nadimpalli, S.K. (2002). Purification and characterization of a galactose-specific lectin from the stem and leaves of *Dolichos lablab* (Indian lablab beans). *Curr Sci* **82(8):**1005-9.

Product References

Bowman, C. C., and Clements, J. D. (2001). Differential biological and adjuvant activities of cholera toxin and *Escherichia coli* heat-labile enterotoxin hydrids. *Infec Immunity* **69(3):**1528-35.

Coukell, B., *et al.* (2004). The Ca^{2+}/calcineurin-regulated cup gene family in *Dictyostelium discoideum* and its possible involvement in development. *Eukaryotic Cell* **3(1):**61-71.

Iglesias, J. L., *et al.* (1982). Purification and properties of a D-galactose/N-acetyl-D-galaxtoasamine-specific lectin from *Erythrina cristagalli*. *Eur J Biochem* **123(2):**247-52.

Matsumoto, J., *et al.* (2001). A novel C-type lectin regulating cell growth, cell adhesion and cell differentiation of the multipotent epithelium in budding tunicates. *Development* **128(17):**3339-47.

Okamoto, K., *et al*. (1998). Contribution of the disulfide bond of the A subunit to the action of *Escherichia coli* heat-labile enterotoxin. *J Bacteriology* **180(6):**1368-74.

Tinker, J. K., *et al.* (2003). Cholera holotoxin assembly requires a hydrophobic domain at the A-B5 interface: mutational analysis and development of an *in vitro* assembly system. *Infec Immunity* **71(7):**4093-101.

Yasuda, K., *et al*. (2004). Efficient and rapid purification of human α-galactosidase A by affinity column chromatography. *Protein Express Purif* **37:**499-506.

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