INSTRUCTIONS

Immobilized Boronic Acid Gel

20244

0601.2

Number	Description
20244	**Immobilized Boronic Acid Gel**, 10mL gel supplied as 50% aqueous slurry with 0.02% sodium azide Support: beaded spherical polyacrylamide with 1800 MW exclusion limit Loading: 100µmol boronate/mL gel Spacer: *m*-aminophenyl group Capacity: ≥ 99% binding and recovery of 110µmol AMP per mL of gel **Storage:** Upon receipt store at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific Immobilized Boronic Acid Gel is an easy-to-use affinity support for purification of nucleotides and other small molecular weight compounds that contain cis-diol groups. The ligand (*m*-aminophenylboronic acid) binds to the cis-diol groups on the sugar portion of nucleotides, forming a reversible five-member ring complex. After washing away non-bound molecules from the sample, the complex can be dissociated and the nucleotide eluted by lowering the pH or by addition of sorbitol.

The polyacrylamide gel used in this product excludes molecules > 1800 MW from entering the internal spaces of the beads, allowing only smaller molecules to interact and bind with the full measure of boronate ligand. Consequently, the gel may not purify glycosylated proteins efficiently, although it has been used successfully for this application.[1] Boronic acid affinity chromatography has been used to isolate ribonucleoside,[2] to purify nucleosidyl peptide,[3] separate ribonucleosidases in tissue extracts,[4] separate RNA and oligoribonucleotide,[5] and determine of the amount of non-enzymatic glycosylation present in peripheral nerve from diabetic and control rats and dogs.[6]

Example Procedure

1. Pack the needed amount of Immobilized Boronic Acid Gel into an empty column (See Related Thermo Scientific Products).
2. Equilibrate/Wash packed gel with 5 column volumes of binding buffer (0.2M ammonium acetate, pH 8.8).
3. Adjust sample pH to 8.5-9.0 and dilute 1:1 with binding buffer.
4. Apply sample to column and allow it to enter and pass through the gel bed. Collect and save flow-through for analysis.
5. Wash column with 2-5 column volumes of binding buffer. Collect and save flow-through for analysis.
6. Elute bound material with 0.1M formic acid, 25mM HCl, or 0.2M sorbitol. Collect small fractions as they emerge from the column, then identify and combine those containing the molecule of interest.
7. Regenerate the column for reuse by washing with 5 column volumes of elution buffer.

Related Thermo Scientific Products

89868	**Pierce Centrifuge Columns, 0.8mL,** 50/pkg
89896	**Pierce Centrifuge Columns, 2mL,** 25/pkg
89897	**Pierce Centrifuge Columns, 5mL,** 25/pkg

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Cited References

1. Londoño, I. and Bendayan, M. (2001) Termporary effects of circulating Amadori products on glomerular filtration properties in the normal mouse. *Am. J Physiol Renal Physiol* **280**:F103-11.
2. Gehrke, C.W., *et al*. (1978) Quantitative HPLC of Nucleosides in Biological Materials. *J of Chromatogr* **150**:455-76.
3. Annamalai, A.E., *et al*. (1979). Purification of nucleosidyl peptides by chromatography on dihydroxyboryl-substituted polyacrylamide and cellulose. *Anal Biochem* **99**:85-91.
4. Olsson, R.A. (1979) Phenyl(dihydroboryl) polyacrylamide beads for chromatography of ribonucleosides in tissue extracts. *J Chromatogr* **176**:239-41.
5. B. Pace and N.R. Pace. (1980). The chromatography of RNA and oligoribonucleotides on boronate-substituted agarose and polyacrylamide. *Anal Biochem* **107**:128-35.
6. Vlassara, H., *et al*. (1981) Nonenzymatic glycosylation of peripheral nerve protein in diabetes mellitus. *Proc Natl Acad Sci* **78(8)**:5190-2.

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.