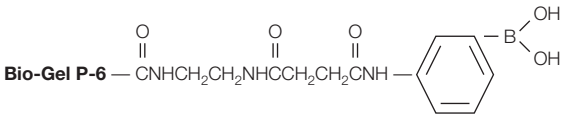
Affi-Gel® Boronate Affinity Gel to Separate Ribonucleotides, Ribonucleosides, Sugars, Catecholamines, and Coenzymes

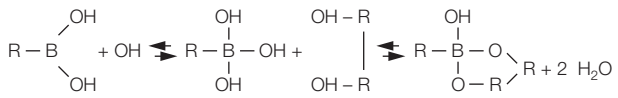
Introduction

Low molecular weight molecules such as nucleotides, nucleosides, catecholamines, coenzymes, and sugars can be separated very efficiently by chromatography on Affi-Gel boronate gel. Affi-Gel boronate gel is a boronate-derivatized polyacrylamide gel with an affinity for adjacent *cis* hydroxyl (*cis*-diol) groups (Figure 1). It effectively separates molecules, such as AMP and cyclic AMP (cAMP), which differ only in the presence or absence of such a group. It also separates *cis*-diol-containing molecules such as cytosine, uridine, and adenosine from one another — all are bound, but their differing affinities permit separate elution (Figure 2A). Bio-Gel® P-6 beads, the gel beads derivatized to make Affi-Gel boronate gel, have a molecular weight exclusion limit of 6,000. The binding capacity of Affi-Gel boronate gel is, therefore, extremely high for low molecular weight compounds.

The development and use of boronate affinity media stems from the observation that boronate forms complexes with sugars possessing coplanar *cis*-diol groups (Boeseken 1949). The structure of the boronate affinity group in Affi-Gel boronate gel is:



The mechanism of binding has been suggested to be reversible formation of a five-member ring (Lorand et al. 1959).



Affi-Gel boronate **Free diol** **Bound diol**

Boronate derivatives on various solid supports have been investigated and applied (Table 1). These derivatives have been variously termed dihydroxy boryl, boric acid gel, and boronic acid gel, all referring to m-aminophenyl boronic acid immobilized to solid supports. Supports have included aminoethyl cellulose, porous glass, methacrylate gel, and Bio-Gel P polyacrylamide gel.

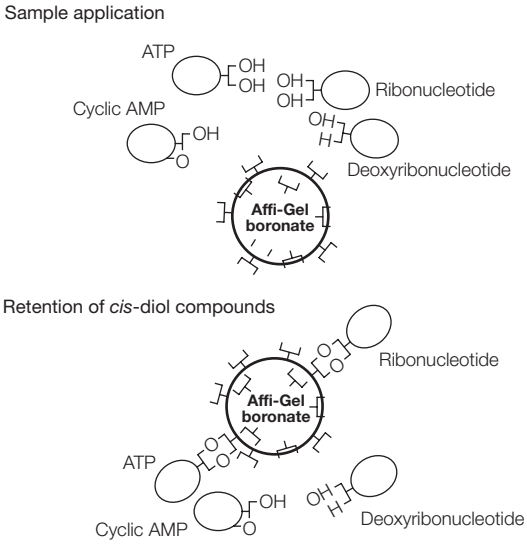


Fig. 1. Separation by affinity for *cis*-diols.

Affi-Gel boronate gel offers a variety of advantages as a support for the applications listed in Table 1:

- High boronate capacity — 1.05 ± 0.15 meq/g
- High capacity for diols — binds 130 µmol sorbitol/ml gel*
- Spherical polyacrylamide matrix — excellent flow rate, resolution, and reproducibility
- Low nonspecific absorption, optimum porosity — low exclusion limit precludes significant binding of high molecular weight molecules, while allowing rapid equilibration and binding of low and medium molecular weight molecules

Preparation and Use

The gel is supplied as dried material and must be hydrated in an appropriate buffer. Buffer choice is dependent upon application (Table 1). Buffers such as Tris should be avoided, since they can adversely affect binding capacity (Schott et al. 1973). The presence of Mg^{2+} may enhance binding. In general, binding is effected at pH >7.5 and elution at pH <6.5. Boric acid, sorbitol, or mannitol can also be used for elution.

* Dr R Hurlbert, personal communication.

Table 1. Applications using boronate affinity media.

Application	Application Buffer	Molecules in V_o	Retained Molecules	Elution Buffer	Support Used	References
Adenylate cyclase assay	HEPES pH 7.5 + $MgCl_2$	cAMP	ATP, AMP, and adenosine	0.05 M NaOAc	Bio-Gel P-150 boronate gel	Hageman and Kuehn 1977
Isolation of catecholamines from urine	0.1 M phosphate pH 7.0 + EDTA	Other urine components and DOPA	Norepinephrine, epinephrine	0.025 N HCl	Boric acid gel (Sigma-Aldrich)	Higa et al. 1977
Modified nucleosides in urine	0.25 M NH_4OAc, pH 8.8	Thymidine, adenine	Pseudouridine	0.1 M HCOOH	Bio-Gel P-2 gel, 200–400 mesh, boronate	Davis et al. 1977 Uziel et al. 1976
Separation of mono- and oligonucleotides	Triethyl ammonium	Deoxyribo-nucleotides	Ribonucleotides	H_2O and others	Dihydroxyboryl methacrylate	Schott et al. 1973
Sugars	0.05 M N-methyl-morpholinium-Cl, pH 7.5 + 1 M NaOAc	Erythritol, adonitol, sucrose, and D-glucose	L-arabinitol, xylitol, D-mannitol, dulcitol, sorbitol, and D-fructose*	Isocratic run, elution buffer same as application buffer	Dihydroxyboryl cellulose	Weith et al., 1970
Assay of ribonucleotide reductase	Tris, $MgCl_2$	dUMP, dCMP	CDP	Citrate	Dihydroxyboryl cellulose Dowex 1(AG) resin	Moore et al. 1974

* In order of increasing retention by support.

Regeneration and Storage

Regeneration of the gel is facilitated by washing with starting buffer if a low-pH elution buffer has been used. If boric acid buffer or diol solutions have been used for elution, the gel must first be washed with 0.1 M acetic acid before reequilibrating with starting buffer.

Hydrated gel can be stored at 4°C under neutral pH conditions for up to 2 months; dry material has excellent long-term stability.

Typical Procedure

Stepwise elution is typical for separation of a ribonucleotide (such as GMP) from a cyclic nucleotide (cGMP) (Figure 2B):

1. Prepare a 1 x 4 cm column of Affi-Gel boronate in 0.1 M HEPES, pH 8.5.
2. Load a 200 µl solution containing 1 µmol each cGMP and GMP in the starting buffer onto the column.
3. Wash the column with 5 column volumes of starting buffer at a flow rate of 1 ml/min and collect the cyclic nucleotide.
4. Elute the ribonucleotide with 0.1 M $NaPO_4$, pH 6.0.

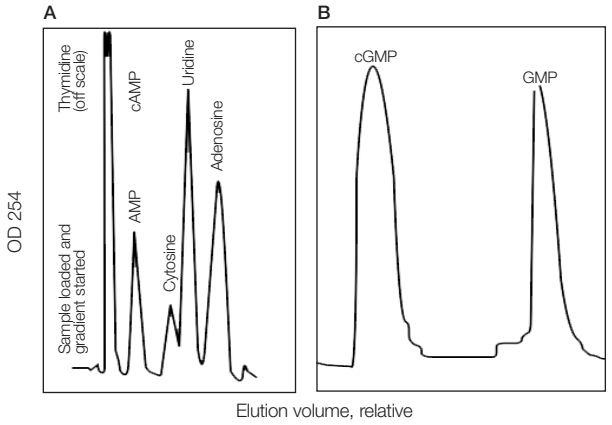


Fig. 2. Examples of separations using Affi-Gel boronate. A, separation of thymidine, cAMP, AMP, adenosine, cytosine, and uridine by a typical gradient elution. A sample containing 0.5 µmol of each molecule was loaded on an equilibrated 0.7 x 45 cm bed of Affi-Gel boronate gel; 0.1 M phosphate was applied after the first peak emerged. The flow rate was 40 ml/hr. B, separation of cGMP and GMP. A sample containing 1.0 µmol cGMP and GMP was loaded onto a 1 x 4 cm column equilibrated with 0.1 M HEPES, pH 8.5. GMP was eluted with 0.1 M $NaPO_4$, pH 6.0, and detected by a UV monitor.

References

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Ordering Information

Catalog #	Description
153-6103	Affi-Gel Boronate Gel, 5 g
153-6104	Affi-Gel Boronate Gel, 50 g

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