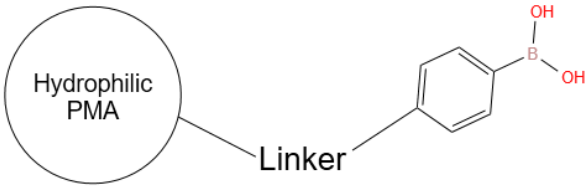
# Boronate-1P Use and Applications

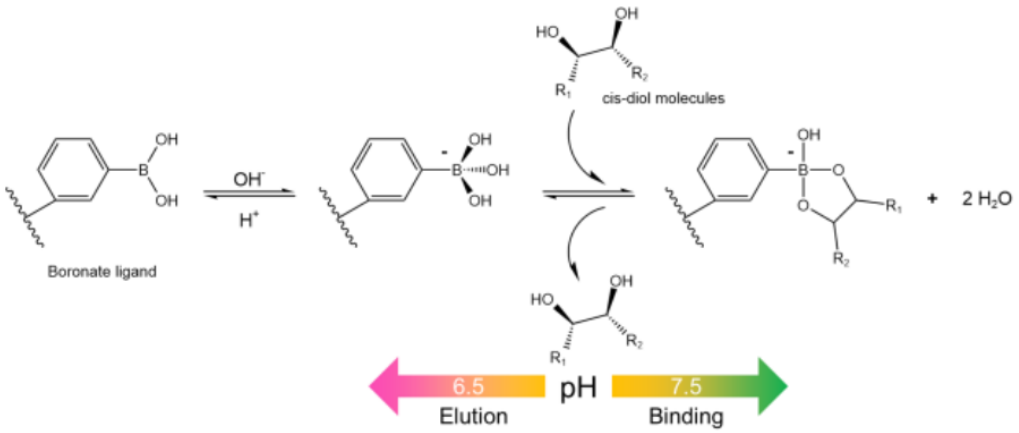
## 1. Description




Chemical structure of Boronate-1P

Boronate-1P is an affinity chromatography medium based on a uniform hydrophilic polymethacrylate matrix with phenylboronic acid functional groups bonded to the surface. It is designed for the purification or analysis of compounds with cis-diol groups, such as glycoproteins, nucleic acids, and sugars. The medium has a particle size of 60 μm and a pore size of 1000 Å. This medium is resistant to pressure and exhibits excellent physicochemical stability. The surface of the medium is highly hydrophilic, which avoids non-specific adsorption with biomolecules.

The affinity interaction between Boronate-1P and cis-diol compounds depends on pH conditions. The mechanism involves the covalent binding of two hydroxyl groups (-OH) of the cis-diol compound molecule with the boronic acid group to form a stable five-membered cyclic ester under alkaline conditions (pH > 7.5). This structure dissociates when the pH is reduced below 6.5, releasing the adsorbed molecules from the medium. $Mg^{2+}$ can enhance the binding capacity of the affinity medium, and molecules bound to the medium can also be eluted with small molecules containing cis-diol structures, such as sorbitol and mannitol.




Working principle of Boronate-1P

**Characteristics:**

- High binding capacity and excellent biocompatibility.
- Rigid matrix can withstand high pressure and flow rates.

- High resolution, high column efficiency, and high recovery rate.
- High batch-to-batch reproducibility.
- Easy to scale up.
- Highly hydrophilic surface.
- Negligible non-specific adsorption.
- Minimal volume change under regular column packing conditions.
- Product supply capacity: >100 L.

## 2. Performance indicators

| Item | Specification |
|---|---|
| Matrix | Hydrophilic polymethacrylate |
| Particle Size | 60 μm |
| Pore Size | 1000 Å |
| Ligand | Phenylboronic acid |
| DBC | 10-30 (μmol sorbitol/mL-resin) |
| Maximum Linear Velocity | 1800 cm/h |
| Operating Temperature | ≤ 40°C |
| pH Range | 2-12 |
| Operating Pressure | ≤ 1 MPa (10 bar) |
| Flow Mobile Phase Compatibility | Compatible with aqueous solutions, water and mixtures of acetonitrile, acetone or methanol, typical buffer systems are: phosphate, acetate and HEPES, MES, buffer salt systems not containing primary amines; The use of buffer solutions with a pH of less than 6.5 or reagents containing glycols may affect adsorption. |
| Long-term Storage Method | Stored in 50% (v/v) ethanol solution. |
| Cleaning and Regeneration | Generally cleaned under conditions of 2-10°C using 3-10 times the column volume of 0.1-1 M NaOH solution. |

## 3. Instructions for Use

### 3.1 Pre-use Cleaning

This product is generally shipped in an aqueous solution containing 20% ethanol and needs to be cleaned

before use. Cleaning can be completed by rinsing with deionized water three times the volume of the medium.

**3.2 Column Packing**

1). Calculate the volume (V) of the chromatography column: V = column cross-sectional area ($\pi r^2$) * column bed height (h), where r is the column inner radius.

2). Shake the packing medium evenly to fully disperse it, forming a uniform slurry. Measure the required volume of the packing medium and transfer it to an appropriate container. After natural sedimentation, pour off the upper aqueous solution, add 3 column volumes (CV) of deionized water, gently mix well, and allow natural sedimentation for about 30 minutes. Pour off the supernatant liquid and repeat this process three times.

3). After pouring off the upper liquid, pour in the column packing buffer (such as 0.5 M NaCl solution), adjust the slurry concentration to 50-70%, mix well, and let it stand for more than 12 hours (overnight).

4). From the prepared slurry, extract the medium containing about 1.05 times the column bed volume, add to the slurry, mix well, and pour it all at once into the chromatography column tube with an appropriately sized screen plate at the bottom, allowing the liquid to flow out and the medium to settle and stabilize.

5). Install a distributor at the top of the chromatography column tube, compact the medium, and connect it to the infusion pump.

6). Flush the column bed with the column packing buffer at twice the working flow rate for 2-3 column volumes. To ensure stability, the height of the distributor can be adjusted during the process to ensure the compactness of the column bed. It is not recommended to use the suction method or rely solely on gravity sedimentation to fill the chromatography column, especially for columns with a bed height exceeding 10 cm.

7). Quality assessment of the chromatography column: Use low molecular weight or non-retained compounds for column efficiency evaluation. Specific operational parameters are as follows:

| **Sample** | **1.0% (v/v) aqueous solution of acetone** | **1 M NaCl** |
|---|---|---|
| Sample Volume | 1.0-5.0% of column bed volume | 1.0-5.0% of column bed volume |
| Mobile Phase | Water or diluted buffer solution | Water or 0.5 M NaCl solution |
| Flow Rate | 180 cm/h | 180 cm/h |
| Detection | 280 nm Ultraviolet Detector | Electric conductivity detector (EC detector) |

| | |
|---|---|
| Qualified standard | Tail factor: 0.8 - 1.5;<br>Column efficiency: ≥ 2000 /m. |

8). Solutions for suboptimal column efficiency: When a tailing peak occurs, solutions include:

- Reducing the slurry concentration.
- Increasing the packing flow rate.
- Extending the static time in the column.

When a fronting peak occurs, the solutions are the opposite of those for a tailing peak.

**3.3 Column Usage**

1). Select and optimize the equilibrium buffer system based on the specific characteristics of the sample to be separated, purified, or analyzed. It is recommended to use a 0.02 M HEPES buffer system containing 0.1-0.5 M NaCl, with a pH > 7.5. A slightly increased ionic strength can help shield ionic non-specific interactions. Additionally, magnesium chloride or calcium chloride can be added to the system to enhance the binding capacity of the packing material.

2). Equilibrate the chromatography column with approximately 5 times the column volume of equilibrium buffer until the conductivity and pH of the effluent remains unchanged and are consistent with the equilibrium buffer.

3). Sample Preparation: Dissolve solid samples in the equilibrium buffer; low-concentration samples can be concentrated by dialysis with the equilibrium buffer; high-concentration samples can be diluted with the equilibrium buffer. Samples with impurities should be filtered to avoid clogging the chromatography column and to extend its service life.

4). Sample Loading: The amount of sample loaded should be determined based on the capacity of the medium and the content of the target in the sample solution; after loading, continue to pump the equilibrium buffer until the baseline is stable.

5). Elution: Choose the elution method based on the characteristics of the sample. Competitive elution generally uses a buffer system of 0.02 M HEPES with the addition of other reagents with high concentrations of cis-diols; moreover, under acidic conditions, the affinity of boronic acid is very weak, so acidic solutions can also be directly used for elution, such as 0.1 M glycine or 0.1 M acetic acid, formic acid, etc., with a pH of 3.0.

6). Cleaning In Place (CIP): Generally, a 0.1-1 M NaOH solution of 3-10 column volumes is used for regeneration. In addition, the cleaning process may cause an increase in the column's back pressure, so it is recommended to use a lower initial flow rate and adjust it appropriately according to the column pressure.

**Note:** During the use, all samples and mobile phases must be filtered with a 0.45 μm pore size filter membrane.