# Protocol

TD-P Revision 1.0

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## Purification of Pepstatin Binding Molecules

### Utilizing Immobilized Pepstatin Agarose Resin

## Introduction

Pepstatin is a hexa-peptide containing statine and a potent inhibitor of various aspartic proteases, such as cathepsin D and E, chymosin, renin, pepsin, HIV proteases and bacterial aspartic proteases. Pepstatin can also be used to purify these macromolecules when used in a column as part of a chromatography system. The resin consists of 6% beaded agarose covalently coupled to pepstatin through a diaminodipropylamine (DADPA) 23 angstrom spacer arm and has a binding capacity of 1-2 mg pepsin per millimeter of settled resin.

## Materials

- Immobilized Pepstatin Agarose Resin (GoldBio Catalog # I-040)

Required, but not supplied:

- Binding Buffer
  - 0.1M citrate, 0.5M sodium chloride, at pH 3.0
- Wash Buffer
  - 0.5M sodium chloride
- Elution Buffer
  - 0.1M sodium bicarbonate, 0.5M sodium chloride, at pH 8.7
- Gravity flow columns
- Sample dialyzed against binding buffer

## Storage/Handling

Immobilized Peptstatin may be shipped at room temperature. Store at 4°C upon receipt; do NOT freeze.

## Method

1. Aliquot 2 ml of the slurry into an appropriate gravity column.

2. Allow the storage buffer to drain out and discard.

3. Equilibrate the resin with 5 ml of the binding buffer.

4. Add the prepared sample to the resin and allow to pass through under gravity.

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5. Wash the column with four resin bed volumes of binding buffer. Repeat four times.

6. Wash the column with four resin bed volumes of wash buffer. Repeat four times.

7. Elute the bound protein with four resin bed volumes of wash buffer six times. Buffer and collect appropriate size fractions (0.5-1 ml). Some proteins may require additional elution buffer volumes.

8. Monitor the elution of proteins by reading the absorbance at 280 nm.

9. Store column by first washing with five resin volumes of water containing 0.05% sodium azide. Store upright at 4°C in deionized water with 0.05% sodium azide.

## Troubleshooting

| Problem | Cause | Solution |
|---|---|---|
| Protein of interest does not bind the resin | The protein does not bind pepstatin | Research pepstatin binding capability and optimal binding conditions. Ensure binding buffer pH is 3.0. |
| | Protein is inactive | Use extraction conditions to maintain proteins activity. |
| Proteins fail to elute | Protein did not bind | Research pepstatin binding capability and optimal binding conditions. Ensure binding buffer pH is 3.0. |
| | Elution not appropriate | Use stronger elution method; use higher salt concentration or higher alkaline conditions |