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A Geno Technology, Inc. (USA) brand name

ρ-Aminobenzamidine Agarose

(Cat. #786-692)

INTRODUCTION

ρ-Aminobenzamidine Agarose primary application is for the removal and/or purification of trypsin-like proteases. ρ-aminobenzamidine (PAB) is a synthetic inhibitor of trypsin-like proteases and has been covalently coupled to 6% cross-linked agarose. ρ-aminobenzamidine agaroses have been used to purify a large range of specific proteins, including serine proteases. The proteins purified include:

- Trypsin
- Thrombin (Bovine)
- Urokinase
- Enterokinase (human)
- Acrosin
- Plasminogen
- Kallikrein
- Prekallikrein
- Collagenase
- Clostripain

For recombinant protein purification, the ρ-Aminobenzamidine Agarose can be used to remove the serine proteases (thrombin and enterokinase) that are used for cleavage of recombinant protein purification tags.
The ρ-Aminobenzamidine Agarose also contains a 6- carbon spacer arm between the ρ-Aminobenzamidine group and the agarose beads, making it suitable for coupling of small proteins and peptides. The long spacer arm minimizes steric hindrance allowing high efficient binding of ligands, including small proteins and peptides.

ITEM(S) SUPPLIED (Cat. # 786-692)

Description	Size
ρ-Aminobenzamidine Agarose	25ml resin

STORAGE CONDITION

Supplied as a suspension in 20% ethanol to maintain activity. It is shipped at ambient temperature. Upon receiving store at 4°C, do not freeze.

PROPERTIES

- 90µm mean particle size
- 45-165µm particle size range
- Spherical, highly cross-linked 6% agarose
- 8-14mg trypsin/ml drained resin binding capacity
- 8µmol ρ-aminobenzamidine/ml drained resin ligand density
- 3-13 pH stability

TRYPSIN-LIKE PROTEASE BINDING PROTOCOL

Additional Items Required Items

- Empty columns
- Coupling/Wash Buffer: 50mM Tris, 0.5M NaCl, pH8.0.
- Elution Buffer: 50mM Glycine pH3.0
- Low pH Wash Buffer: 0.1M Sodium acetate, 0.5M NaCl, pH4.5

Protocol

1. Allow the reagents to reach room temperature.
   NOTE: *The binding to the resin may be temperature sensitive for some proteins. If low or no protein binding is observed, perform the procedure at 4°C.*
2. Gently resuspend the ρ-Aminobenzamidine Agarose by inverting the bottle 2-3 times. Once a homogeneous slurry is formed, remove an appropriate volume of the slurry and place in a suitable size column.
3. Remove the caps from the column and transfer the column to a suitable collection tube. Briefly centrifuge at 1,000g for 1 minute to remove the storage buffer. Discard the flow-through.
4. Immediately, add 1 column volume (CV) of Coupling/Wash Buffer to the resin bed, centrifuge at 1,000xg for 1 minute and discard the flow-through. Repeat this step once.
5. Replace the bottom cap on the column and add approximately 1-2CV sample containing the proteases to the equilibrated resin. Do not exceed the binding capacity of the resin.
   NOTE: *It is recommended that the samples are filtered through a 0.45µm filter to remove large particles before loading on to the resin.*
   OPTIONAL*: Remove an aliquot of the protein solution to be used as 'starting material' to determine coupling efficiency, if required.*
6. Seal the column and incubate for 15-60 minutes at room temperature with end-over-end mixing.
7. Remove the caps from the column and transfer the column to a suitable collection tube. Centrifuge at 1,000g for1 minute. Save the flow-through to assay binding efficiency.
   NOTE*: The coupling efficiency is determined by measuring and comparing the concentration of the protein/peptide in 'starting material' and in the supernatant.*
8. Wash the coupled agarose with 1CV of Coupling/Wash Buffer. Centrifuge at 1,000g for 1 minute. Repeat once.
9. Elute the protein with 1CV Elution Buffer and repeat until the elution protein concentrations reach a baseline as monitored by UV absorbance or a suitable protein assay.

REMOVAL OF THROMBIN PROTOCOL

The protease thrombin is routinely used in the removal of recombinant protein tags, such as GST, due to its small protein recognition domain. The thrombin can interfere with downstream applications so its removal is often required.

Additional Items Required Items

- Empty columns
- Coupling/Wash Buffer: 20mM sodium phosphate, 150mM sodium chloride, pH7.5
- Elution Buffer: 20mM ρ-aminobenzamidine in Coupling/Wash Buffer

Protocol

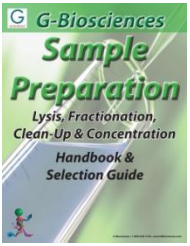
1. Allow the reagents to reach room temperature.
   NOTE: *The binding to the resin may be temperature sensitive for some proteins. If low or no protein binding is observed, perform the procedure at 4°C.*
2. Gently resuspend the ρ-Aminobenzamidine Agarose by inverting the bottle 2-3 times. Once a homogeneous slurry is formed, remove an appropriate volume of the slurry and place in a suitable size column.
3. Remove the caps from the column and transfer the column to a suitable collection tube. Briefly centrifuge at 1,000g for 1 minute to remove the storage buffer. Discard the flow-through.
4. Immediately, add 1 column volume (CV) of Coupling/Wash Buffer to the resin bed, centrifuge at 1,000xg for 1 minute and discard the flow-through. Repeat this step once.
5. Replace the bottom cap on the column and add approximately 1-2CV thrombin treated protein sample to the equilibrated resin. Do not exceed the binding capacity of the resin.
   NOTE: *It is recommended that the samples are filtered through a 0.45µm filter to remove large particles before loading on to the resin.*
   OPTIONAL*: Remove an aliquot of the protein solution to be used as 'starting material' to determine coupling efficiency, if required.*
6. Seal the column and incubate for 15-60 minutes at room temperature with end-over-end mixing.
7. Remove the caps from the column and transfer the column to a suitable collection tube. Centrifuge at 1,000g for1 minute. The flow through contains the protein of interest without thrombin contamination.
8. Wash the coupled agarose with 1CV of Coupling/Wash Buffer. Centrifuge at 1,000g for 1 minute. Repeat once.
9. Elute the bound thrombin with 1CV Elution Buffer and repeat until the elution protein concentrations reach a baseline as monitored by UV absorbance or a suitable protein assay.

REGENERATION PROTOCOL

1. Wash the column with 2-3CV Coupling/Wash Buffer.
2. Wash the column with 2-3CV Low pH Wash Buffer.
3. Repeat the cycle of steps 1 and 2 three times total.
4. Wash the column with 2-3CV Coupling/Wash Buffer supplemented with a suitable preservative.

RELATED PRODUCTS

Download our Sample Preparation Handbook.



http://info.gbiosciences.com/complete-protein-sample-preparation-handbook

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