Epoxy-Activated Agarose (Dry Form)

For covalent binding of primary amine, thiol or hydroxyl group containing ligands to a solid support

(Cat. #786-1221, 786-1222)

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INTRODUCTION

Epoxy-Activated Agarose is preactivated resin used for affinity chromatography. Epoxy-Activated Agarose comprise of high density epoxy groups that form covalent bonds with ligands containing amine groups, thiol or hydroxyl groups.

Epoxide-Acivated Agarose is prepared by immobilization of oxiranes such as 1,4-butanediol diglycidyl ether onto matrix. This pre-activated resin provide hydrophilic 12 atom spacer arm which enables binding of small ligand molecules such as amino acids, monosaccharide, peptides and carbohydrates.

Due to long hydrophilic spacer arm and binding of several nucleophiles ($-NH_2$, -SH, -OH) containing ligands, Epoxy-Activated Agarose find widespread application in affinity chromatography involving purification of antigen, antibody, lectin, glycoproteins etc.

Since Epoxy-Activated Agarose has limited stability in aqueous media, it it supplied as dry form. In addition it is recommended that it is used quickly after rehydration.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-1221	Epoxy-Activated Agarose (Dry Form)	5 g
786-1222	Epoxy-Activated Agarose (Dry Form)	15 g

STORAGE CONDITION

Supplied as dry form to maintain activity. It is shipped at ambient temperature. Upon receiving store at 4°C.

FEATURES

- Resin Active group: epoxy
- Resin Active group density: 20-40 µM/ ml hydrated resin.
- Group to be coupled: $-NH_2$, -OH or -SH
- 45-165µm particle size range
- Spherical, highly cross-linked 6% agarose
- Resin spacer arm: 12 atom hydrophilic spacer arm
- Coupling conditions: pH 7.5-8.5 for thiol ligands, pH 9-10 for amine ligands and pH 11-13 for hydroxyl ligands at 4 to 37°C depending upon ligand stability for 16 hrs
- pH stability: 2-13, ligand dependent
- 1 gm of dry resin swells to 5-6 ml.
- Storage: 2- 8°C, desiccated

ADDITIONAL ITEMS REQUIRED

- Empty columns (Cat. # 786-810, 786-724)
- Coupling Buffers:
    1. **Phosphate Buffer**: 0.1M phosphate buffer+0.15 NaCl, pH 7.2-7.5 for coupling of ligands to the matrix via their thiol groups.
    2. **Carbonate Buffer**: 0.1 M carbonate buffer + 0.15 M NaCl, pH 9-11 for coupling of peptides and proteins to the matrix through their amine groups
    3. **Carbonate Buffer**: 0.1 M carbonate buffer + 0.15 M NaCl, pH>11 adjusted with NaOH. Used for coupling of sugar and carbohydrates via their hydroxyl groups.

  NOTE: *The pH of coupling buffer is dependent on the target ligand[1].*
- Wash Buffer PBS, pH7.4.
- Carbohydrates, Protein, peptide or other ligands with primary amines or hydroxyl groups or thiol groups depending upon what molecule need to be affinity purified.
- Quenching or Blocking Buffer: 1M ethanolamine , pH8

IMPORTANT INFORMATION

Before planning an optimum coupling reaction, ensure that parameters listed below are met.

1. **Coupling Buffer/Solution pH:** Most of the coupling reaction occurs in pH range 9-13. However there is exception when coupling ligands via their thiol groups as they are highly reactive with epoxides and thus needs buffer system closer to physiological pH range of 7.5-8.5. Proteins and peptides are normally coupled via their amine groups in pH range 9-11 depending upon stability of the ligand.
2. **Coupling Buffer/Solution composition:** No amine-containing, thiol group or hydroxyl group containing buffers should be used as coupling buffers as they bind to the resin thus interfere with binding of ligand to the resin.
   Coupling can be done in distilled water, carbonate buffers, phosphate buffers or borate buffers. Sodium hydroxide can be used to make solutions of hig pH
3. **Coupling reaction temperature and time:** Most of the coupling is carried out in range 20°C to 40°C, however temperature can go as low as 4°C depending upon ligand stability. Direct contact of resin with heat should be avoided. Use water bath shaker instead. The coupling reaction time decreases with increase in temperature. The reaction time also depends upon pH of Coupling Buffer and properties of ligand.
4. **Ligand concentration used for coupling:** Generally for big ligands add 100 to 400 µmol/ ml drained gel or 5-10 times concentration of active groups. For small ligands add ≥ 200 µmol/ml drained gel.

PROTOCOL

Preparation of Ligand for coupling to Epoxy-Activated Agarose

Dissolve the protein or ligand to be coupled in the Coupling Buffer.

NOTE: *If the protein is in buffer that interfere with coupling then buffer exchange to Coupling Buffer can be done using G-Biosciences G-Trap™ GT-600 Desalting Columns (Cat. # 786-1023) or SpinOUT™ GTs (Cat. # 786-170). Alternatively, dialyze the sample against Coupling Buffer using G-Biosciences' Tube-O-Dialyzer™.*

Preparation of resin for coupling of ligands

1. Soak an appropriate amount of preactivated resin in distilled water such as 1 g of dry preactivated resin in 10 ml of double distilled water.
   NOTE: *1 g resin swell to 5-6 ml*
2. Gently wash the resin thrice with distilled water. The resin is ready for coupling.
   NOTE: *Either use filter funnel for washing or centrifuge for 1 min at 600-1000 g and gently remove supernatant.*

Coupling of ligands to the Epoxy-Activated Agarose

1. Add two volumes of ligand solution to one volume of swelled resin and mix gently. Mix gently overnight (~16 hrs) at 4°C-37°C depending upon temperature stability of ligand on end-over-end mixer or a rocker
   NOTE: *Do **not** use magnetic stir bar as it may grind agarose beads.*
2. Remove the unreacted ligand by washing the resin five times with Wash Buffer.
3. Add two gel volumes of 1 M ethanolamine, pH8 (Quenching or Blocking Buffer) to the resin and incubate for 4 hrs to overnight at 4°C.
4. Wash the resin with 0.1 M acetate buffer pH4.0 containing 0.5M NaCl followed by wash with 0.1 M Tris-HCl buffer pH8 containing 0.5M NaCl.
5. Repeat step 4 two times.
6. For immediate use, load the resin on column and equilibrate with binding buffer used for the affinity chromatography.
   NOTE: *Store the resin in Wash Buffer (PBS) at 2-8°C with 0.02% sodium azide if the resin is not used immediately*.

REFERENCES

1. Sundberg, L. and Porath, J. (1974). Preparation of adsorbants for biospecific affinity chromatography. Attachment of group-containing ligands to insoluble polymers by means of bifunctional oxiranes. J. Chrom 90, 87-98.

RELATED PRODUCTS

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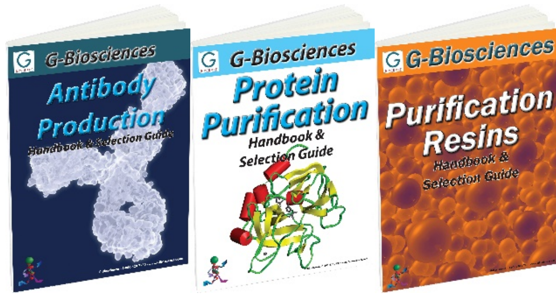

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