

SepSphere™ Alprenolol Agarose

White beads suspension in water.

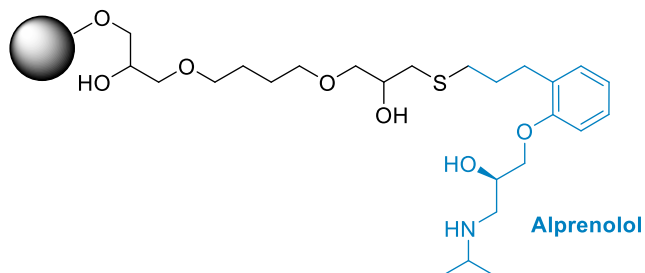
1 mL, 5 mL, or 25 mL settle down beads.

Product Numbers: [CM29101-1ML](#),
[CM29101-5ML](#), [CM29101-25ML](#)

Product Description

Alprenolol is an antagonist for β_1 and β_2 adrenergic receptors. Alprenolol agarose affinity chromatography was first introduced by Lefkowitz R. J. in 1979 (References 1 and 2) for the purification of erythrocyte β -adrenergic receptor based on ligand-receptor interactions. It is now routinely used for the purification of functionally active β_1 and β_2 -adrenergic receptors. CellMosaic's SepSphere™ Alprenolol Agarose is composed of Agarose 4B Beads linked to alprenolol via a thiol ether linkage.

Agarose



Application of the Product

- Purification of β -adrenergic receptor.

Chemical Information

- **Chemical Name:** Alprenolol Agarose
- **Ligand:** Alprenolol
- **Immobilization Method:** Immobilized through the reaction of the double bond of alprenolol and S-alkyl-thiosulfate agarose.
- **Matrix:** Agarose 4B (Sources: GE Sciences)
- **Spacer:** 12 atoms.
- **Particle Size:** 45 – 165 μm
- **Loading Capacity:** 1 ~ 3 μmole per mL of settled beads by thiol assay (check the certificate of analysis for specific lot).
- **Storage Temp:** 2 - 8°C (Compatible preservative may be added to prevent algae growth).

References

1. Shorr, R., G.; Lefkowitz, R. J.; Caron, M. G. Purification of the β -adrenergic receptor. *J. Bio. Chem.* **1981**, 256(11), 5820-5826.
2. Caron, M. G.; Srinivasan, Y.; Pitha, J.; Kocielek, K.; Lefkowitz, R. J. Affinity Chromatography of the β -adrenergic Receptor *J. Bio. Chem.* **1979**, 254(8), 2923-2927.

General Protocol of β -Adrenergic Receptor Purification

Items needed (not provided):

High salt buffer: 20 mM Tris buffer, pH 7.4, 500 mM NaCl

Low salt buffer: 20 mM Tris buffer, pH 7.4, 100 mM NaCl

Glycerol and n-Dodecyl-beta-Maltoside (DM)

Empty Columns

1. Preparation of Resin: Transfer alprenolol agarose resin into a centrifuge tube. Equilibrate resin with high-salt buffer containing 10% glycerol, 0.05% DM. Centrifuge at 2000 x g for 2 minutes. Discard the supernatant. Repeat this step one more time.

Tip: The amount of resin you will use depends on the amount of receptor you have in your crude mixture. The capacity of alprenolol- Agarose resin used here is about 0.25 mg receptor per 1 mL of settled resin. You can use 2 mL per 0.25 mg of receptor in this step.

2. Sample Application: Add half amount of alprenolol agarose resin from **Step 1** to the crude receptor solution. Incubate or nutate at room temperature (RT) for 1 hour. Centrifuge at 2000 x g for 2 minutes. Transfer the supernatant to a new centrifuge tube and add the rest of the resin from **Step 1**. Incubate or nutate at RT for 1 hour.

3. Washing: Combine the resins from **Step 2** and transfer to an empty column. Wash the resin six times with 1 column volume (CV) of ice-cold high-salt buffer containing 0.05% DM, six times with 1 CV of ice-cold low-salt buffer containing 0.05% DM, and six times with 1 CV of ice-cold high-salt buffer containing 0.05% DM.

Tip: if the total volume of resin is too much (>1 mL), resin can be washed by centrifugation at 2000 x g for 2-4 minutes. Do not centrifuge resin dry.

4. Elution: The resin is suspended in 2 CV of high-salt buffer containing 0.05% DM and 2 mM ($\pm$) alprenolol. Incubate or nutate at RT for 1 hour. Centrifugation at 2000 x g for 5 minutes. Collect the flow-through and label it as elution. Repeat once and combine the elute.

Tip: DM concentration can be reduced to 0.02% in this elution buffer; For crystallization purpose, (-) alprenolol should always be used instead of (+) alprenolol.