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Technical Information about Rp- & Sp-8-AEA-cAMPS-Agarose

Gels for affinity chromatography of cAMP-phosphodiesterases and protein kinases

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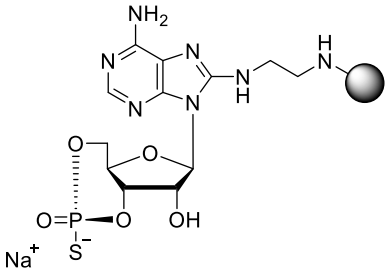
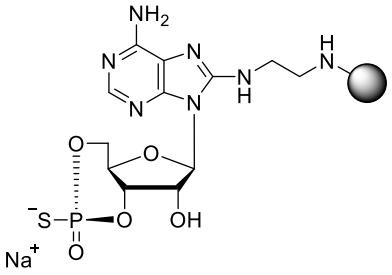


Rp-8-AEA-cAMPS Agarose



Sp-8-AEA-cAMPS Agarose

BIOLOG Cat. No.: **A 007** **A 008**

Name: Rp-/ Sp-8-(2-Aminoethylamino)adenosine- 3', 5'- cyclic monophosphorothioate agarose

Description: The PKA inhibitor Rp-cAMPS (Cat. No. A 002) and the corresponding agonistic Sp-cAMPS (Cat. No. A 003) are immobilized to agarose via a short spacer at position 8 of the adenine nucleobase. Synthesis and distribution is protected by patent DE 3802865.4, licensed to BIOLOG Life Science Institute.

Properties of Rp-8-AEA-cAMPS-Agarose: The ligand of this gel represents an immmobilized form of the cyclic AMP antagonist Rp-cAMPS. Free Rp-cAMPS was found to prevent dissociation of protein kinase A I & II catalytic from regulatory subunits and thus to inhibit the cAMP signal pathway. In addition, Rp-cAMPS is not hydrolyzed by mammalian phosphodiesterases.
Both properties make this analogue to be an interesting ligand in affinity chromatography, especially when trying to purify protein kinase A holoenzyme, cyclic nucleotide-dependent phosphodiesterases or other cAMP binding proteins.
Immobilized Rp-cAMPS can also be used as a stable general ligand with reduced affinity for various nucleotide binding proteins since PDE degradation, the first metabolic step necessary in cyclic nucleotide metabolism, is blocked.
Immobilized Rp-cAMPS is also available with a longer aminohexylamino spacer (Rp-8-AHA-cAMPS-Agarose, Cat. No. A 012); for other spacer types, attachment positions or gels please inquire.

Properties of Sp-8-AEA-cAMPS-Agarose: Just as its corresponding Rp-version Sp-8-AEA-cAMPS-Agarose can be used for affinity chromatography of protein kinases, phosphodiesterases or other cyclic nucleotide-dependent proteins. However, since the free Sp-cAMPS is an activator of cAMP kinases the holoenzyme complex should dissociate on this column material.
In addition, free Sp-cAMPS is only extremely slowly hydrolyzed by mammalian phosphodiesterases and the spacer in position 8 further reduces enzymatic hydrolysis, so the gel can be used for phosphodiesterase purification and isozyme pattern analysis as well. Except for the Catabolite Activator Protein (CAP) the Sp-isomers of phosphorothioates show higher affinity for cAMP receptors compared to the Rp-analogues. Immobilized Sp-cAMPS can also be used as a stable general ligand with reduced affinity for various nucleotide binding proteins since PDE degradation, the first metabolic step necessary in cyclic nucleotide metabolism, is blocked.
Immobilized Sp-cAMPS is also available with a longer aminohexylamino spacer (Sp-8-AHA-cAMPS-Agarose, Cat. No. A 013); for other spacer types, attachment positions or gels please inquire.

If the target protein is absorbed too strongly on common cAMP agaroses, both gels can be used as alternatives due to the reduced binding affinity of phosphorothioate-modified cyclic nucleotides to most receptors. Also, the column can be useful for purification of cGMP receptors in case the protein sticks too tightly to a corresponding gel with immobilized cGMP.

Specification: Suspension in 30 mM Na_2HPO_4 buffer (pH 7). Ligand density: approximately 6 µmol/ml of settled gel. UV: λ_{max} 273 nm/suspension in glycol.

Purity: The purity of each ligand to be attached is better than 98% (HPLC).

Stability and Storage: 8-AEA-cAMPS agarose has sufficient stability for chromatography at room temperature and does not need special care during handling or shipment. Nevertheless for longer storing periods the gel should be kept in the refrigerator at +4 - +8°C since desulfurization yielding immobilized cAMP can occur slowly (about 0.1 % week/20°C). **Storage buffer should contain 0.1% sodium azide for prevention of microbial growth.**

BIOLOG Life Science Institute, Bremen, Germany Phone: 49 (0) 421 591355 Fax: 49 (0) 421 5979713 e-mail: service@biolog.de

Chromatography: After equilibration with about 10 column volumes of starting buffer the affinity column is loaded with the protein solution, e.g. at 50-200 µl/min. In order to elute other nucleotide-dependent proteins bound unspecifically, the column is washed, e.g. with 1 mM 5'-AMP. For elution of E. coli nucleoside diphosphate kinase it is recommended to wash the column with 10 mM ADP/20mM $MgCl_2$.
Elution of the target protein is performed by a cyclic nucleotide gradient up to 40 mM. For elution of phosphodiesterases it could be advisable to use the highly hydrolysis-resistant Sp-cAMPS or the stable Rp-cAMPS. For protein kinase holoenzyme elution the gradient should be made with Rp-cAMPS.
Suitable buffer systems for your special application have to be tested, but phosphate should be not optimal since one essential affinity interaction of cyclic nucleotides towards their target receptors is the cyclic phosphate.
Regeneration can be achieved by a combination of up to 100 mM cAMP and buffer salts or 8 M urea.

Toxicity and Safety: Please keep in mind that the *in vivo* properties of these compounds are not sufficiently characterized up to now. Avoid skin contact or ingestion and allow only trained personnel to work with it.
Our products are designed, developed and sold for research purposes only. They are intended for *in vitro* and non-human *in vivo* laboratory applications. Any other use requires approval of health authorities.
Not for drug, household or related uses!

Selected References for 8-AEA-cAMP-Agarose:

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