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Technical Information about Rp- & Sp-8-AET-cGMPS-Agarose

Gels for affinity chromatography of cGMP-phosphodiesterases and protein kinases

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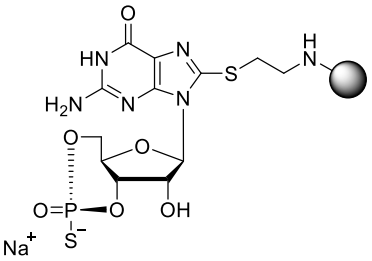
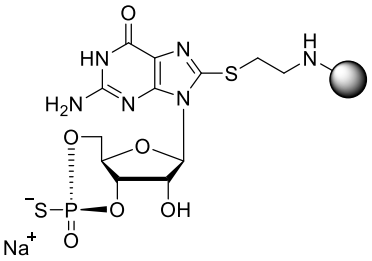


Rp-8-AET-cGMPS Agarose



Sp-8-AET-cGMPS Agarose

BIOLOG Cat. No.: **A 009** **A 010**

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

Description: The PKG inhibitor Rp-cGMPS and the corresponding agonistic Sp-cGMPS are immobilized to agarose via a short spacer at position 8 of the adenine nucleobase.

Properties of Rp-8-AET-cGMPS-Agarose: The ligand of this gel represents an immmobilized form of the cyclic GMP antagonist Rp-cGMPS. Free Rp-cGMPS was found to prevent dissociation of protein kinase A I & II catalytic from regulatory subunits and thus to inhibit the cGMP signal pathway. In addition Rp-cGMPS 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 G, cyclic nucleotide-dependent phosphodiesterases or other cGMP binding proteins.
Immobilized Rp-cGMPS 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.
For other spacer types, attachment positions or gels please inquire.

Properties of Sp-8-AET-cGMPS-Agarose: Just as its corresponding Rp-version Sp-8-AET-cGMPS agarose can be used for affinity chromatography of protein kinases, or other cyclic nucleotide-dependent proteins.
Since free Sp-cGMPS is only extremely slowly hydrolyzed by mammalian phosphodiesterases and the spacer in position 8 further reduces enzymatic hydrolysis, the gel can be used for phosphodiesterase purification and isozyme pattern analysis as well. Generally, the Sp-isomers of phosphorothioates seem to have higher affinity for the corresponding cGMP receptors compared to the Rp-analogues. Immobilized Sp-cGMPS 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.
For other spacer types, attachment positions or gels please inquire.

If the target protein is absorbed too strongly on common cGMP 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 cAMP receptors in case the protein sticks too tightly to a corresponding gel with immobilized cAMP.

Specification: Suspension in 30 mM Na_2HPO_4 buffer (pH 7). Ligand density: approximately 5 µmol/ml of settled gel.

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

Stability and Storage: 8-AET-cGMPS 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.**

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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 unspecifically bound, the column is washed, e.g. with 1 mM 5'-GMP. 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-cGMPS or the stable Rp-cGMPS.
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 cGMP 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-AET-cGMP-Agarose:

Dills, W.L.; Beavo, J.A.; Bechtel, P.J.; Myers, K.R.; Sakai, L.J., Krebs, E.G., *Biochemistry* **15**, 3724 - 3770 (1976): "Binding of Adenosine 3',5'-Monophosphate Dependent Protein Kinase Regulatory Subunit to Immobilized Cyclic Nucleotide Derivatives"

Dills, W.L.; Beavo, J.A.; Bechtel, P.J.; Krebs, E.G., *Biochem. Biophys. Res.Commun*., **62**, 70 - 77 (1975): "Purification of Rabbit Skeletal Muscle Protein Kinase Regulatory Subunit Using Cyclic Adenosine- 3':5'- Monophosphate Affinity Chromatography"

References for phosphorothioate-modified cyclic nucleotide agarose:

Van Bemmelen, M.X.P, Ph.D. Thesis, University of Bremen, 1993, Bremen, Germany

Iwitzki, F.; Van Bemmelen, M.X.P.; Genieser, H.-G.; Jastorff, B., Proc. Internatl. Conf. Second Messengers and Phosphoprot., Glasgow, UK 1992: “New Affinity Materials for Cyclic AMP Binding Proteins“

Iwitzki, F.; Van Bemmelen, M.X.P.; Genieser, H.-G.; Jastorff, B., Proceedings of the 3rd Swedish-German Workshop on Nucleic Acid Synthesis, Structure and Function, Uppsala, Sweden, 1992: "cAMP Analogues Designed for Affinity Chromatography"

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