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Technical Information about Rp-2'-AHC-cGMPS-Agarose

Gel for affinity chromatography of cGMP-dependent protein kinases and phosphodiesterases

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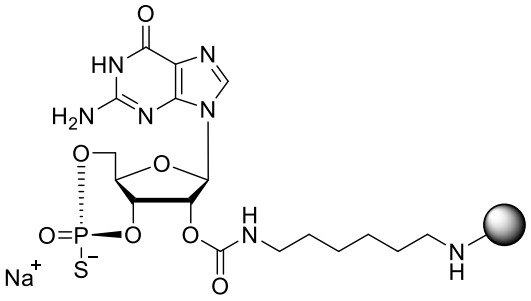

Abbreviation: **Rp-2'-AHC-cGMPS-Agarose**

BIOLOG Cat. No.: A 051

Description: In Rp-2'-AHC-cGMPS-Agarose the Rp- isomer of 2'- (6- aminohexylcarbamoyl)guanosine- 3', 5'- cyclic monophosphorothioate has been immobilized as an affinity ligand by the hydroxysuccinimide technique. Rp-2'-AHC-cGMPS is an analogue of the parent second messenger cyclic GMP in which one of the two exocyclic oxygen atoms in the cyclic phosphate moiety is modified by sulfur. Equatorial thio substitution leads to the R- isomer, while axial modification yields the corresponding S- compound. The suffix "p" indicates that R/S nomenclature refers to phosphorus. The 2'- hydroxy group carries the aminohexylcarbamoyl spacer for attachment.

Properties: Rp-2'-AHC-cGMPS-Agarose is a gel for affinity chromatography of various cGMP-responsive proteins, especially those which tolerate modification of the 2'- ribose hydroxyl group, such as certain phosphodiesterases. Due to its phosphorothioate modification the affinity to most cGMP receptor proteins are weaker, so desorption procedure should be milder. In addition, destruction of the ligand by phosphodiesterases is blocked.

Related Products: This type of gel is also available with the corresponding Sp- isomer (Sp-2'-AHC-cGMPS-Agarose, Cat. No. A 052) and the normal, sulfur-free ligand (2'-AHC-cGMP-Agarose, Cat. No. A 059). For immobilized cGMP or cAMP with other spacer types, attachment positions, different immobilization techniques or on other gels please inquire.

Specification: Ligand density: approximately 6 µmol/ml of settled gel. UV: λ_{max} 252 nm/suspension in glycol.

Stability and Storage: Rp-2'-AHC-cGMPS-Agarose has sufficient stability for chromatography at ambient 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°. **Storage buffer should contain 0.1 % sodium azide for prevention of microbial growth.**

Chromatography: After equilibration with about 10 column volumes of starting buffer the affinity column is loaded with the protein solution at 50 - 100 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 phosphorothioate-modified analogs or PDE inhibitors.
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.

Selected References for cGMP Affinity Chromatography:

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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"

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