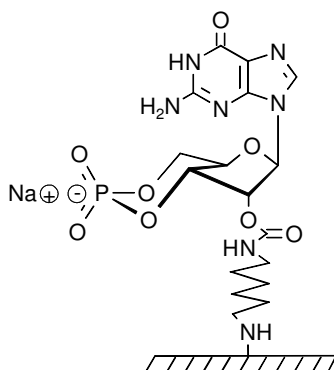


Technical Information about 2'-AHC-cGMP-Agarose

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

Update: October 11, 2007 TR



Abbreviation: **2'-AHC-cGMP-Agarose**

BIOLOG Cat. No.: A 059

Description: In 2'-AHC-cGMP-Agarose 2'- (6- amino-hexyl-carbamoyl)guanosine- 3', 5'- cyclic monophosphate has been immobilized as an affinity ligand by the hydroxysuccinimide technique.

Properties: 2'-AHC-cGMP-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.

Related Products: This type of gel can be offered with PDE-stable phosphorothioate modification Rp-/Sp-2'-AHC-cGMPS as well. For immobilized cGMP or cAMP with other spacer types, attachment positions, different immobilization techniques or on other gels please inquire. The free ligand is offered separately as well (Cat. No. A 048).

Specification: Suspension in 30 mM Na₂HPO₄ buffer (pH 7), containing 0.1 % sodium azide as preservative. Ligand density: approximately 6 µmol/ml of settled gel. UV: λ_{max} 252 nm/ suspension in glycol

Stability and Storage: 2'-AHC-cGMP-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 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, 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 MgCl₂.

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 this compound 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!

P.t.o.

Selected References for cGMP Affinity Chromatography:

Kim, E.; Park, J.M., *J. Biochem. Mol. Biol.*, **36**, 299 - 304 (2003): "Identification of Novel Target Proteins of Cyclic GMP Signaling Pathways Using Chemical Proteomics"

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"

Scholten, A.; Poh, M.K.; van Veen, T.A.; van Breukelen, B.; Vos, M.A.; Heck, A.J., *J. Proteome Res.*, **6**, 1435 - 1447 (2006): "Analysis of the cGMP/cAMP Interactome Using a Chemical Proteomics Approach in Mammalian Heart Tissue Validates Sphingosine Kinase Type 1-interacting Protein as a Genuine and Highly Abundant AKAP"