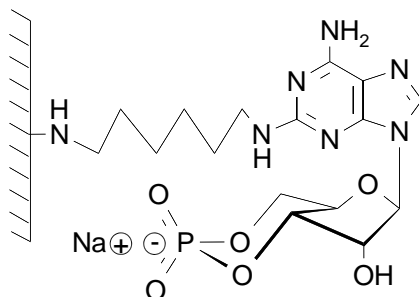


## Technical Information about 2-AHA-cAMP-Agarose

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

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**Abbreviation:** 2-AHA-cAMP-Agarose

**BIOLOG Cat. No.:** A 054

**Description:** In 2-AHA-cAMP-Agarose 2-(6-aminohexylamino)adenosine-3', 5'-cyclic monophosphate has been immobilized as an affinity ligand by the hydroxysuccinimide technique.

**Properties:** 2-AHA-cAMP-Agarose is a gel for affinity chromatography of various cAMP-responsive proteins such as protein kinases or phosphodiesterases.

**Related Products:** For immobilized cAMP or cGMP 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 053).

**Specification:** Suspension in 30 mM Na<sub>2</sub>HPO<sub>4</sub> buffer (pH 7). Ligand density: approximately 6 µmol/ml of settled gel. UV: λ<sub>max</sub> 258 nm/suspension in glycol.

**Stability and Storage:** 6-AH-cAMP-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, 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'-AMP. For elution of E. coli nucleoside diphosphate kinase it is recommended to wash the column with 10 mM ADP/20mM MgCl<sub>2</sub>.

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.

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 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!**

### Selected References for 2-AHA-cAMP-Agarose:

Donaldson, L.; Meier, S.; Gehring, C., *Cell Commun. Signal*, **14**(1):10 (2016): "The Arabidopsis Cyclic Nucleotide Interactome"

Donaldson, L.; Meier, S., *Methods Mol. Biol.*, **1016**, 155 - 173 (2013): "An Affinity Pull-Down Approach to Identify the Plant Cyclic Nucleotide Interactome"

Hanke, S.E.; Bertinetti, D.; Badel, A.; Schweinsberg, S.; Genieser, H.-G.; Herberg, F.W., *N. Biotechnol.*, **28**, 294 - 301 (2011): "Cyclic Nucleotides as Affinity Tools: Phosphorothioate cAMP Analogues Address Specific PKA Subproteomes"

Margarucci, L.; Roest, M.; Preisinger, C.; Bleijerveld, O.B.; van Holten, T.C.; Heck, A.J.R.; Scholten, A., *Mol. Biosyst.*, **7**, 2311 - 2319 (2011): "Collagen Stimulation of Platelets Induces a Rapid Spatial Response of cAMP and cGMP Signaling Scaffolds"

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"

Iwitzki, F., PhD Thesis, University of Bremen, Bremen, Germany, 1993.

#### References for cAMP Affinity Chromatography:

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"