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Technical Information about 8-(6-Aminohexylamino)-cAMP

Site-selective activator of cAMP-depending protein kinase, precursor for fluorescence labelling and ligand for affinity chromatography of cyclic nucleotide binding proteins

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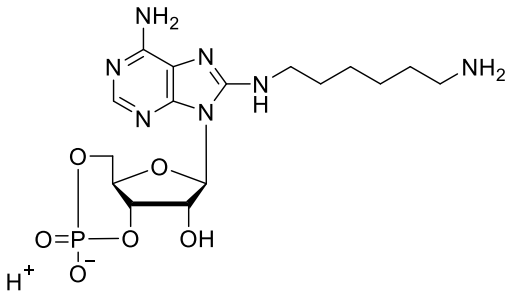

Abbreviation: **8-AHA-cAMP**

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat. No.
$C_{16}H_{26}N_7O_6P$ (acid)	[39824-30-1]	443.4	λ_{max} 273 nm / ε 17000 / pH 7	A 011

Name: 8- (6- Aminohexylamino)adenosine- 3', 5'- cyclic monophosphate

Description: 8-AHA-cAMP is an analogue of the natural signal molecule cyclic AMP where the hydrogen in position 8 of the nucleobase is replaced by an aminohexylamino group.

Properties:

- **Activator of protein kinase A (PKA),**
- **site selective for site B of PKA I and hence a suitable partner for synergistic activation by pairs of analogues with opposite site selectivity,**
- **suitable for immobilization as a ligand for affinity chromatography and, e.g. , for binding of fluorescent dyes,**
- **high metabolic stability towards cyclic nucleotide-responsive phosphodiesterases.**

8-AHA-cAMP is a selective activator of cAMP-dependent protein kinase, which is hardly metabolized by mammalian cyclic nucleotide-responsive phosphodiesterases. Due to its site selectivity, it is frequently used as a partner for selective stimulation of PKA type I by synergistic pairs of cAMP analogues. The free terminal amino group (separated from the nucleotide by a hexyl spacer) is suitable for coupling to gels for affinity chromatography and for binding of various labels, e.g. fluorescent dyes. 8-AHA-cAMP as well as the corresponding phosphorothioate analogues, Rp- and Sp-8-AHA-cAMPS, are available from BIOLOG also as ligands already immobilized to agarose (Cat. Nos. A 028 / A 012 / A 013).

Application: If 8-AHA-cAMP is combined with an analogue which selects site A of PKA I (e.g. 8-PIP-cAMP, Cat. No. P 002), type I of PKA is selectively activated. Please ask for the special corresponding technical leaflet (No. TI1001) on this topic.

Specification: Crystallized or lyophilized solid. Equal concentrations of 8-AHA-cAMP can appear very different in volume due to sensitivity of the lyophilized form to humidity. The compound can even contract to small volume droplets. Normally the product is located in the conical bottom of the tube. Micromolar quantities are determined by UV at λ_{max}.

Purity: Typical analysis is better than 98% (HPLC / UV / 273 nm). The product is not sterile and has not been tested for endotoxins.

Stability and Storage: 8-AHA-cAMP is chemically stable under conditions of biological systems and media. Nevertheless, we recommend that the compound should be stored in the freezer, for longer storage periods preferably in freeze-dried form.

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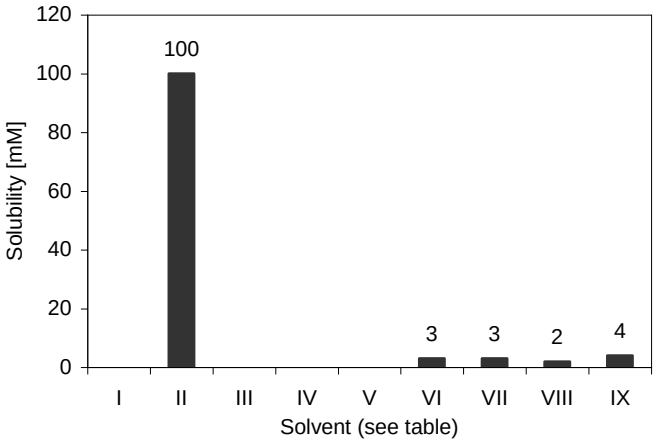
Solubility: Detailed information on the solubility of 8-AHA-cAMP in water and various buffers are listed in the solubility chart below. Concentrations have been determined at ambient temperature and can be considered as minimum concentrations usually obtainable, however, slight batch-to-batch variations cannot be ruled out. When opening the tube please make sure that no substance is lost within the cap. Please rinse tube walls carefully and preferably use ultrasonic or vortex to achieve total and uniform mixing.

No.	Solvent	Solubility [mM]
I	H_2O	0
II	DMSO	100
III	DMF	0
IV	Ethanol 96%	0
V	Methanol	0
VI	PBS, pH 7.4	3
VII	100 mM Na_2HPO_4, pH 7.0	3
VIII	25 mM Hepes/NaOH, pH 7.2	2
IX	25 mMTris/HCl, pH 7.4	4



Toxicity and Safety: Since cyclic AMP has multiple tasks in every organism, it is possible that cAMP analogues will interfere with many cell regulation processes *in vivo*. However, due to the rather small quantities to work with no health hazards have been reported. Nevertheless 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 handle the product.
Our products are designed, developed and sold for research purposes only. They are intended for *in vitro* and nonhuman *in vivo* laboratory applications. Any other use requires approval of health authorities.
Not for drug, household or related uses!

References for 8-AHA-cAMP:

Lolicato, M.; Nardini, M.; Gazzarrini, S.; Möller, S.; Bertinetti, D.; Herberg, F.W.; Bolognesi, M.; Martin, H.; Fasolini, M.; Bertrand, J.A.; Arrigoni, C.; Thiel, G.; Moroni, A., *J. Biol. Chem.*, **286**, 44811 - 44820 (2011): "Tetramerization Dynamics of C-terminal Domain Underlies Isoform-specific cAMP Gating in Hyperpolarization-activated Cyclic Nucleotide-gated Channels"

Hanke, S.E.; Bertinetti, D.; Badel, A.; Schweinsberg, S.; Genieser, H.-G.; Herberg, F.W., *N. Biotechnol.*, Epub ahead of print (2010): "Cyclic Nucleotides as Affinity Tools: Phosphorothioate cAMP Analogues Adress Specific PKA Subproteomes"

Bertinetti, D.; Schweinsberg, S.; Hanke, S.E.; Schwede, F.; Bertinetti, O.; Drewianka, S.; Genieser, H.-G.; Herberg, F.W., *BMC Chem Biol*, **9** (2009): "Chemical tools selectively target components of the PKA system"

Moll, D.; Prinz, A.; Gesellchen, F.; Drewianka, S.; Zimmermann, B.; Herberg, F.W., *J. Neural. Transm.*, **113**, 1015 - 1032 (2006): "Biomolecular Interaction Analysis in Functional Proteomics"

Jensen, B.O.; Selheim, F.; Doeskeland, S.O.; Gear, A.R.L.; Holmsen, H., *Blood*, **104**, 2775-2782 (2004): "Protein Kinase A Mediates Inhibition of the Thrombin-Induced Platelet Shape Change by Nitric Oxide"

Kopperud, R.; Krakstad, C.; Selheim, F.; Doeskeland, S. O., *FEBS Lett.*, **546**, 121 - 126 (2003):" cAMP Effector Mechanisms. Novel Twists for an 'Old' Signaling System"

Mutafova-Yambolieva, V.N.; Smyth, L.; Bobalova, J., *Cardiovasc. Res.*, **57**, 217 - 224 (2003): "Involvement of Cyclic AMP-mediated Pathway in Neural Release of Noradrenaline in Canine Isolated Mesenteric Artery and Vein"

Singh, A.K.; Tasken, K.; Walker, W.; Frizzell, R.A.; Watkins, S.C.; Bridges, R.J.; Bradbury, N.A., *Am. J. Physiol.*, **275**, C562 - C570 (1998):" Characterization of PKA Isoforms and Kinase-dependent Activation of Chloride Secretion in T84 Cells"

Torgersen, K.M.; Vaage, J.T.; Levy, F.O.; Hansson, V.; Rolstad, B.; Tasken, K., *J. Biol. Chem.*, **272**, 5495 - 5500 (1997): "Selective Activation of cAMP-Dependent Protein Kinase Type I Inhibits Rat Natural Killer Cell Cytotoxicity"

Dienhart, M.K.; Downs, S.M., *Zygote*, **4**, 129 - 137 (1996): "Cyclic AMP Reversal of Hypoxanthine-Arrested Preimplantation Mouse Embryos is EDTA-Dependent"

Oestenstad, B.; Harboe, M.; Lea, T., *Eur. J. Immunol.*, **24**, 2150 - 2154 (1996): "Differential Effects of Cyclic Adenosine 3', 5'-monophosphate on T Cell Cytotoxicity"

Harris, F.M.; Wilkins, A.C.R.; Bayliss, M.A.; Brenton, A.G.; Games, D.E.; Newton, R.P., Langridge, J.I.; Walton, T.J., Intl. Sympos. Appl. Mass Spec. Health Sci. Barcelona, Spain, 1995: "Mass Spectrometric Analysis of Novel Effectors of Cyclic Nucleotide-dependent Protein Kinases"

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Downing, G.J.; Poisner, A.M., *Am. J. Physiol*., **267**, E 954 - E 960 (1994): "cAPK Mediates Placental Renin Secretion Stimulated by ß-Adrenoceptor Activation"

Skalhegg, B.S.; Landmark, B.F.; Døskeland, S.O.; Hansson, V.; Lea, T.; Jahnsen, T., *J. Biol. Chem*., **267**, 15707 - 15714 (1992): "Cyclic AMP-dependent Protein Kinase Type I Mediates the Inhibitory Effects of 3',5'- Cyclic Adenosine Monophosphate on Cell Replication in Human T Lymphocytes"

Døskeland, S.O.; Boe, R.; Bruland, T.; Vintermyr, O.C.; Jastorff, B.; Lanotte, M., Method. Surveys Biochem. & Anal. **21**, Cell Signalling: Experimental Strategies, E. Reid et al. (eds.). Royal Society Chem., Cambridge, UK 1991, Redwood Press Ltd.: "Criteria Used to Judge That a Cellular Response is Mediated by Cyclic AMP"

Lanotte, M.; Riviere, J.B.; Hermouet, S.; Houge, G.; Vintermyr, O.K.; Gjertsen, B.T.; Døskeland, S.O., *J. Cell. Physiol*., **146**, 73 - 80 (1991): "Programmed Cell Death (Apoptosis) Is Induced Rapidly and With Positive Cooperativity by Activation of Cyclic Adenosine Monophosphate-Kinase I in a Myeloid Leukemia Cell Line"

Steinberg, R.S., Gorman, K.B.; Øgreid, D.; Døskeland, S.O.; Weber, I.T., *J. Biol. Chem*., **266**, 3547 - 3553 (1991): "Mutations That Alter the Charge of Type I Regulatory Subunitand Modify Activation Properties of Cyclic AMP-dependent Protein Kinase from S49 Mouse Lymphoma Cells"

Dostmann, W.R.G.; Taylor, S.S.; Genieser, H.-G.; Jastorff, B.; Døskeland, S.O.; Øgreid, D., *J. Biol. Chem*., **265**, 10484 - 10491 (1990): "Probing the Cyclic Nucleotide Binding Sites of cAMP-Dependent Protein Kinase I and II With Analogs of Adenosine 3',5'-Cyclic Phosphorothioates"

Øgreid, D.; Ekanger, R.; Suva, R.H.; Miller, J.P.; Døskeland, S.O., *Eur. J. Biochem*., **181**, 19 - 31 (1989): "Comparison of the Two Classes of Binding Sites (A andB) of Type I and Type II Cyclic-AMP-dependent Protein Kinases by Using Cyclic Nucleotide Analogs"

Woodford, T.A.; Correll, L.A.; McKnight, G.S.; Corbin, J.D., *J. Biol. Chem*., **264**, 13321 - 13328 (1989): "Expression and Characterization of Mutant Forms of the Type I Regulatory Subunit of cAMP-dependent Protein Kinase"

Francis, S.H.; Noblett, B.D.; Todd, B.W.; Wells, J.N.; Corbin, J.D., *Mol. Pharmacol*., **34**, 506 - 517 (1988): "Relaxation of Vascular and Tracheal Smooth Muscle by Cyclic Nucleotide Analogs That Preferentially Activate Purified cGMP-Dependent Protein Kinase"

Øgreid, D.; Døskeland, S.O.; Gorman, K.B.; Steinberg, R.A., *J. Biol. Chem*., **263**, 17397 - 17404 (1988): "Mutations That Prevent Cyclic Nucleotide Binding to Binding Sites A or B of Type I Cyclic AMP - dependent Protein Kinase"

Steinberg, R.A.; Russell, J.L.; Murphy, C.S.; Yphantis, D.A., *J. Biol. Chem*., **262**, 2664 - 2671 (1987): "Activation of Type I Cyclic AMP- dependent Protein Kinases with Defective Cyclic AMP-binding Sites"

Øgreid, D.; Ekanger, R.; Suva, R.H.; Miller, J.P.; Sturm, P.; Corbin, J.D; Døskeland, S.O., *Eur. J. Biochem*., **150**, 219 - 227 (1985): "Activation of Protein Kinase Isozymes by Cyclic Nucleotide Analogs Used Singly or in Combination"

Beebe, S.J.; Holloway, R.; Rannels, S.R.; Corbin, J.D., *J. Biol. Chem*., **259**, 3539 - 3547 (1984): "Two Classes of cAMP Analogs Which Are Selective for the Two Different cAMP-binding Sites of Type II Protein Kinase Demonstrate Synergism When Added Together to Intact Adipocytes"

Grivennikov, I.A.; Petukhov, S.P.; Bulargina, T.V.; Gulyaev, N.N.; Severin, E.S., *Biokhimiya* **49**, 1395 - 1406 (1984): "cAMP-dependent Protein Kinase from Pigeon Breast Muscle. Isolation of the Regulatory Subunit by the Method of Affinity Chromatography and Study of the Topography of the cAMP-Binding Site Using cAMP Analogs"

Døskeland, S.O.; Øgreid, D.; Ekanger, R.; Sturm, P.A.; Miller, J.P.; Suva, R.H., *Biochemistry* **22**, 1094 - 1101 (1983): "Mapping of the Two Intrachain Cyclic Nucleotide Binding Sites of Adenosine Cyclic 3', 5'- Phosphate Dependent Protein Kinase I"

Van Sande, J.; Lefort, A.; Beebe, S.; Roger, P.; Perret, J.; Corbin, J.; Dumont, J.E.**,** *Eur. J. Biochem*., **183**, 699 - 708 (1989): "Pairs of cyclic AMP Analogs, that are Specifically Synergistic for Type I and Type II cAMP-dependent Protein Kinases, Mimic Thyrotropin Effects on the Function, Differentiation Expression and Mitogenesis of Dog Thyroid Cells**"**

Øgreid, D.; Døskeland, S.O.; Miller, J.P., *J. Biol. Chem*., **258**, 1041 - 1049 (1983): "Evidence That Cyclic Nucleotides Activating Rabbit Muscle Protein Kinase I Interact with Both Types of cAMP Binding Sites Associated with the Enzyme"

Robinson-Steiner, A.M.; Corbin, J.D., *J. Biol. Chem*., **258**, 1032 - 1040 (1983): "Probable Involvement of Both Intrachain cAMP Binding Sites in Activation of Protein Kinase"

Corbin, J.D.; Rannels, S.R.; Flockhart, D.A.; Robinson-Steiner, A.M.; Tigani, M.C.; Døskeland, S.O.; Suva, R.H.; Miller, J.P., *Eur. J. Biochem*., **125**, 259 - 266 (1982): "Effect of Cyclic Nucleotide Analogs on Intrachain Site 1 of Protein Kinase Isozymes"