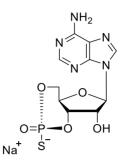


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# **Technical Information about Rp-cAMPS**

## PDE resistant inhibitor of cAMP-dependent protein kinases type I and II

Update: June 08, 2017 HJ



## Abbreviation:

Rp-cAMPS

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat. No.
C <sub>10</sub> H <sub>11</sub> N <sub>5</sub> O <sub>5</sub> PS⋅Na	[73208-40-9]	367.3	$\lambda_{max}$ 259 nm / $\epsilon$ 15200 / pH 7	A 002 S

Name: Adenosine- 3', 5'- cyclic monophosphorothioate, Rp- isomer (Rp-cAMPS)

**Description:** Rp-cAMPS is an analogue of the natural signal molecule cyclic AMP in which the equatorial one of the two exocyclic oxygen atoms in the cyclic phosphate moiety is replaced by sulfur. The suffix "p" indicates that R/S nomenclature refers to phosphorus.

Bulk Supply: Rp-cAMPS can be offered in multigram quantities at extremely competitive prices. Please ask for a corresponding quotation.

#### **Properties:**

- Competitive inhibitor of cyclic AMP-dependent protein kinase I and II 1,2

- metabolic stability towards mammalian cyclic nucleotide- responsive phosphodiesterases <sup>6</sup>

- discriminates between protein kinase A (antagonist) and some other cyclic AMP receptors, e.g. channels or CAP <sup>3, 8</sup> (agonist)

- membrane-permeant for several systems (for improved permeability more lipophilic analogues e.g. Rp-8-Br-cAMPS are recommended

**Specification:** Crystallized sodium salt. Please keep in mind that equal amounts of the compound may look different in volume depending on humidity. Rp-cAMPS is hygroscopic and tends to form a transparent film on the bottom of the tube. Micromolar quantities are determined by UV at 258 nm. Other salt forms of Rp-cAMPS are available upon request.

**Purity:** Typical analysis is > 98% for the triethyl ammonium salt (Economy Grade) and > 99% for the sodium salt (High Purity Grade) by HPLC / UV / 258 nm. The product is not sterile and has not been tested for endotoxins.

Caution: Since even minor impurities of cyclic AMP or the agonistic diastereomer Sp-cAMPS (0.2%) can already activate protein kinase A and compete with the antagonistic effect of the Rp- isomer, it is imperative to work with a strictly pure compound, especially concerning cyclic nucleotide contaminants. Cyclic AMP interference is not so important when working with cell cultures, since cAMP itself has very low membrane permeability and, in addition, would be metabolized immediately by both intracellular and external serum cyclic nucleotide-dependent phosphodiesterases.

Traces of the activator Sp-cAMPS, however, are fatal since it effectively competes with Rp-cAMPS and is only extremely slowly degraded by PDE. Another reason also demands for a pure reagent: Some cyclic AMP binding proteins other than kinases, such as some cyclic nucleotide-gated ion channels or the CAP protein, are activated by Rp-cAMPS. Thus, pure Rp-cAMPS can distinguish between protein kinase A and these receptors, but a contaminated reagent will yield misinformation.

Therefore, BIOLOG's Rp-cAMPS is strictly checked for absence of activators such as Sp-cAMPS or cyclic AMP (< 0.05% when packed). Analysis of Rp-cAMPS from other sources, however, showed that this is not common practice.

**Stability and Storage:** Rp-cAMPS has sufficient stability at room temperature and does not need special care during handling or shipment. Nevertheless, we recommend that the compound should be stored in the freezer, for longer storage periods preferably in freeze-dried form, since cyclic AMP can be formed by oxidation processes.

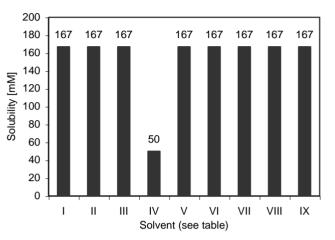
**Solubility:** Detailed information on the solubility of Rp-cAMPS in water and various buffers are listed in the solubility chart below. Concentrations have been tested at ambient temperatures and can be considered as minimum concentrations obtainable. When



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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]
Ι	H <sub>2</sub> O	167
Ш	DMSO	167
III	DMF	167
IV	Ethanol 96%	50
V	Methanol	167
VI	PBS, pH 7.4	167
VII	100 mM Na <sub>2</sub> HPO <sub>4</sub> , pH 7.0	167
VIII	25 mM Hepes/NaOH, pH 7.2	167
IX	25 mMTris/HCl, pH 7.4	167



**Application:** Experience shows that applicable concentrations of Rp-cAMPS depend on the type of biosystem, its membrane properties and kinase content. A main application for Rp-cAMPS is to eliminate the first messenger-stimulated phosphorylation by cyclic AMP-dependent protein kinase. For this purpose preincubation (e.g. 20 min.) is important since the production of intracellular cyclic AMP initiated by a first messenger is much faster than the antagonist can penetrate the membrane when given extracellularly. Since Rp-cAMPS is hydrolytically stable in mammalian and many other systems there is no danger of degradation during incubation periods.

**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!

**Selected References:** Since its first synthesis by F. Eckstein, Göttingen/Germany, there have been more than 500 papers published with Rp-cAMPS, and it is impossible to list them all. However, since we were the first to offer this structure commercially, we have quite a lot of data and experience with it. Please ask for a search in our data base for articles relevant for your field. For an extended reference list please refer to our website <u>http://www.biolog.de</u>.

Rothermel, J.D.; Jastorff, B.; Botelho, L.H.P.; *J. Biol. Chem.*, **259**, 8151 - 8155 (1984): "Inhibition of Glucagon-Induced Glycogenolysis in Isolated Rat Hepatocytes by the Rp-Diastereomer of Adenosine Cyclic 3',5'-Phosphorothioate"

Botelho, L.H.P.; Rothermel, J.D.; Coombs, R. V.; Jastorff, B.; *Methods Enzymol.*, **159**,159 - 172 (1988) : "cAMP Analog Antagonists of cAMP Action"

Scholübbers, H.-G.; Van Knippenberg, P.H.; Baraniak, J.; Stec, W.J.; Morr, M.; Jastorff, B.; *Eur. J. Biochem.*, **138**, 101 - 109 (1984): "Investigations on Stimulation of Lac Transcription in Vivo in Escherichia Coli by cAMP Analogs. Biological Activities and Structure-Activity Correlations"

Scheinman, S.J.; Stec, W.J.; Coulson, R., Miner. *Electrolyte Metab.*, **11**, 85 - 90 (1985): "Effects of (Sp)- and (Rp)-Adenosine Cyclic 3',5' Phosphorothioates on Electrolyte Excretion by the Isolated Perfused Rat Kidney"

Van Haastert, P.J.M.; Kesbeke, F.; Konijn, T.M.; Baraniak, J.; Stec, W.; Jastorff, B.; *Bioact. Mol.*, **3**, 469 - 483 (1987): "(Rp)-cAMPS, an Antagonist of cAMP in Dictyostelium Discoideum"

Erneux, C.; Miot, F., *Methods Enzymol.*, **159**, 520 - 530 (1988): "Cyclic Nucleotide Analogs Used to Study Phosphodiesterase Catalytic and Allosteric Sites"

Dostmann, W.R.G.; Taylor, Susan 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"

Gjertsen, B.T.; Mellgren, G.; Otten, A.; Maronde, E.; Genieser, H.-G.; Jastorff, B.; Vintermyr, O.K.; McKnight, G.S.; Doeskeland, S.O., *J. Biol. Chem.*, **270**, 20599 - 20607 (1995): "Novel (Rp)-cAMPS Analogs as Tools for Inhibition of cAMP-Kinase in Cell Culture"

Kramer, R.H.; Tibbs, G.R., *J. Neuroscience*, **16**, 1285 - 1293 (1996): "Antagonists of Cyclic Nucleotide-gated Channels and Molecular Mapping of their Site of Action"

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"



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Oestreich, E.A.; Wang, H.; Malik, S.; Kaproth-Joslin, K.A.; Blaxall, B.C.; Kelley, G.G.; Dirksen, R.T.; Smrcka, A.V., *Am. Soc. Biochem. Mol. Biol.* [Epub ahead of print] (2006): "Epac-mediated Activation of Phospholipase C-epsilon Plays a Critical Role in Beta-adrenergic Receptor Dependent Enhancement of Ca<sup>2+</sup> Mobilization in Cardiac Myocytes"

Brown, R.L.; Strassmeier, T.; Brady, J.D.; Karpen, J.W., *Curr. Pharmaceut. Design*, **12**, 3597 - 3613 (2006): "The Pharmacology of Cyclic Nucleotide-gated Channels: Emerging from the Darkness"

Meves, H., Curr. Neuropharmacol., 4, 41 - 57 (2006): "The Action of Prostaglandins in Ion Channels"

Scott, S.-P.; Shea, P.W.; Dryer, S.E., *Biochemistry*, **46**, 9417 - 9431 (2007): "Mapping Ligand Interactions with the Hyperpolarization Activated Cyclic Nucleotide Modulated (HCN) Ion Channel Binding Domain Using a Suluble Construct"

Borland, G.; Smith, B.O.; Yarwood, S.J., *Brit. J. Pharmacol.*, **158**, 70 - 86 (2009): "Epac Proteins Transduce Diverse Cellular Actions of cAMP"

Banales, J.M.; Masyuk, T.V.; Gradilone, S.A.; Masyuk, A.I.; LaRusso, N.F., *Hepatology*, **49**, 160 - 174 (2009): "The cAMP Effectors Epac and Protein Kinase A (PKA) are Involved in the Hepatic Cystogenesis of an Animal Model of Autosomal Recessive Polycystic Kidney Disease (ARPKD)"

Werner, K.; Schwede, F.; Genieser, H.-G.; Geiger, J.; Butt, E., *Naunyn Schmiedebergs Arch. Pharmacol.*, **384**, 169 - 176 (2011): "Quantification of cAMP and cGMP Analogs in Intact Cells: Pitfalls in Enzyme Immunoassays for Cyclic Nucleotides"