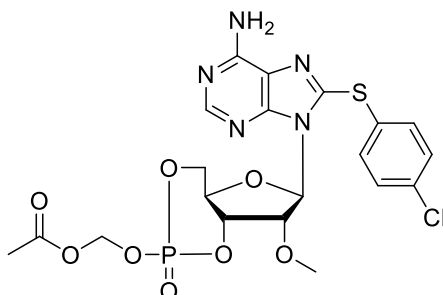


Technical Information about 8-(4-Chlorophenylthio)-2'-O-Me-cAMP-AM

Membrane-permeant, metabolically activatable stimulator of Epac

Update: January 19, 2021 HU



Abbreviation: 8-pCPT-2'-O-Me-cAMP-AM / 8-CPT-2'-O-Me-cAMP-AM

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat. No.
C ₂₀ H ₂₁ ClN ₅ O ₈ PS	[1152197-23-3]	557.9	λ _{max} 282 nm / ε 16000 / pH 7	C 051

Name: *para*-Chlorophenylthio-2'-O-methyladenosine-3', 5'-cyclic monophosphate, acetoxymethyl ester (8-pCPT-2'-O-Me-cAMP-AM) or 8-(4-chlorophenylthio)-2'-O-methyladenosine-3', 5'-cyclic monophosphate, acetoxymethyl ester (8-CPT-2'-O-Me-cAMP-AM)

Description: 8-pCPT-2'-O-Me-cAMP-AM is an analogue of the natural signal molecule cyclic AMP in which the hydrogen in position 8 of the heterocyclic nucleobase is replaced by the lipophilic 4-chlorophenylthio moiety, and the ribose 2'-hydroxy group has been methylated. In addition the polar cyclic phosphate is masked by an acetoxymethyl group.

Properties: The acetoxymethyl group of 8-pCPT-2'-O-Me-cAMP-AM masks the charged polar phosphate and thus makes the molecule highly membrane-permeant. Inside the cell esterases release the more polar 8-pCPT-2'-O-Me-cAMP (Cat. No. C 041), which is a potent and specific agonist of exchange factors directly activated by cAMP (Epac or cAMP-GEF), a newly discovered receptor for cyclic AMP.

Since a free 2'-ribose hydroxyl group in cyclic AMP is essential for stimulation of cAMP-dependent protein kinase (PKA), the methylated structure of 8-pCPT-2'-O-Me-cAMP is an extremely poor PKA activator and allows for specific discrimination between both signalling pathways.

Phosphate tris(acetoxymethyl)ester, PO₄-AM₃ (our Cat. No. P 030), is recommended as control reagent in 8-pCPT-2'-O-Me-cAMP-AM applications to test for side effects of enzymatically released acetic acid and formaldehyde, two metabolites with potential biological functions.

Specification: Micromolar quantities are determined by UV at λ_{max}.

Purity: Typical analysis is better than 97% (HPLC / UV / 282 nm) for the mixture of equatorial and axial isomers. The product is not sterile and has not been tested for endotoxins.

Solubility/Application: Due to its rather high lipophilicity, the solubility of 8-pCPT-2'-O-Me-cAMP-AM in water or buffers is limited (e.g. PBS, pH 7.2: ≥ 50 μM; 50 mM Tris, pH 7.2: ≥ 50 μM). We suggest to use a small amount of anhydrous organic solvent such as anhydrous DMSO or DMF for preparation of 1 - 10 mM stock solutions to be applied directly to the cell culture medium. In some cases, especially at high concentrations (> 50 μM), Pluronic® F-127 (Molecular Probes) can be useful to facilitate solubilization in water or physiological media. Please keep in mind that due to the high potency of 8-pCPT-2'-O-Me-cAMP-AM relatively low concentrations (1 nM - 50 μM) should be sufficient, and be sure to check for DMSO/DMF tolerance in your system. Since 8-pCPT-2'-O-Me-cAMP-AM is bioactivated by esterases, application to cell cultures should be performed without serum supplements (even heat-inactivated serum still contains active esterases!) in the media for at least 15 minutes. Otherwise, serum esterases may strongly reduce the cell-loading efficacy. Please rinse tube walls carefully and preferably use ultrasonic or vortex to achieve total and uniform mixing.

Stability and Storage: 8-pCPT-2'-O-Me-cAMP-AM is sufficiently stable to be shipped at ambient temperature, however, it should be stored in the freezer (-20°C necessary, -80°C recommended). Please note that aqueous solutions are rather labile and should be freshly prepared immediately before use. Stock solutions in anhydrous DMSO or DMF should be relatively stable when stored frozen at -20°C to -80°C.

Toxicity and Safety: Since cyclic AMP has multiple tasks in every organism it is very likely that lipophilic 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 for 8-pCPT-2'-O-Me-cAMP-AM:

Parnell, E.; Palmer, T.M.; Yarwood, S.J., *Trends Pharmacol. Sci.*, **36**, 203 – 214 (2015): " The Future of EPAC-targeted Therapies: Agonism versus Antagonism"

Henquin, J.-C., Nenquin, M., *Endocrinology*, **155**, 3274 - 3287 (2014): "Activators of PKA and Epac Distinctly Influence Insulin Secretion and Cytosolic Ca²⁺ in Female Mouse Islets Stimulated by Glucose and Tolbutamide"

Takahashi, T.; Shibasaki, T.; Takahashi, H.; Sungawara, K.; Ono, A.; Inoue, N.; Furuya, T.; Seino, S., *Sci. Signal.*, **6**, ra94 (2013): "Antidiabetic Sulfonylureas and cAMP Cooperatively Activate Epac2A"

Chepurny, O.G.; Bertinetti, D.; Diskar, M.; Leech, C.A.; Afshari, P.; Tsalkova, T.; Cheng, X.; Schwede, F.; Genieser, H.-G.; Herberg, F.W.; Holz, G.G., *Mol. Endocrinol.*, **27**, 1267-1282 (2013): "Stimulation of Proglucagon Gene Expression by Human GPR119 Enteroendocrine L-Cell Line GLUTag"

Almahariq, M.; Tsalkova, T.; Mei, F.C.; Chen, H.; Zhou, J.; Sastry, S.K.; Schwede, F.; Cheng, X., *Mol. Pharmacol.*, **83**, 122 - 128 (2013): "A Novel EPAC Specific Inhibitor Suppresses Pancreatic Cancer Cell Migration and Invasion"

Dzhura, I.; Chepurny, O.G.; Leech, C.A.; Roe, M.W.; Dzhura, E.; Xu, X.; Lu, Y.; Schwede, F.; Genieser, H.-G.; Smrcka, A.V.; Holz, G.G., *Islets*, **3**, 121 - 128 (2011): "Phospholipase C-epsilon Links Epac2 Activation to the Potentiation of Glucose-stimulated Insulin Secretion from Mouse Islets of Langerhans"

Tsalkova, T.; Mei, F.C.; Cheng, X., *PLoS One*, **7(1)**, e30441 (2011): "A Fluorescence-Based High-Throughput Assay for the Discovery of Exchange Protein Directly Activated by Cyclic AMP (EPAC) Antagonists"

Chepurny, O.G.; Kelley, G.G.; Dzhura, I.; Leech, C.A.; Roe, M.W.; Dzhura, E.; Li, X.; Schwede, F.; Genieser, H.-G.; Holz, G.G., *Am. J. Physiol.-Endocrinol. Metabol.*, **298**, 622 – 633 (2010): "PKA-dependent Potentiation of Glucose-stimulated Insulin Secretion by Epac Activator 8-pCPT-2'-O-Me-cAMP-AM in Human Islets of Langerhans"

Idevall-Hagren, O.; Barg, S.; Gylfe, E.; Tengholm, A., *J. Biol. Chem.*, **285**, 23007 - 23018 (2010): "cAMP Mediators of Pulsatile Insulin Secretion from Glucose-stimulated Single Beta-cells"

Gerlo, S.; Verdood, P.; Kooijman, R., *J. Interferon Cytokine Res.*, **30**, 883 - 891 (2010): "Modulation of Cytokine Production by Cyclic Adenosine Monophosphate Analogs in Human Leukocytes"

Dzhura, I.; Chepurny, O.G.; Kelley, G.G.; Leech, C.A.; Roe, M.W.; Dzhura, E.; Afshari, P.; Malik, S.; Rindler, M.J.; Xu, X.; Lu, Y.; Smrcka, A.V.; Holz, G.G., *J. Physiol.*, **588**, 4871 - 4889 (2010): "EPAC2-dependent Mobilization of Intracellular Ca²⁺ by Glucagon-like Peptide-1 Receptor Agonist Exendin-4 is Disrupted in Beta-Cells of Phospholipase C-Epsilon Knockout Mice"

Gaudy, A.M.; Clementi, A.H.; Campbell, J.S.; Smrcka, A.V.; Mooney, R.A., *J. Biol. Chem.*, **285**, 41356 - 41365 (2010): "Suppressor of Cytokine Signaling-3 is a Glucagon-Inducible Inhibitor of PKA Activity and Gluconeogenic Gene Expression in Hepatocytes"

Leech, C.A.; Dzhura, I.; Chepurny, O.G.; Schwede, F.; Genieser, H.-G.; Holz, G.G., *Islets*, **2**, 72 - 81 (2010): "Facilitation of Beta-Cell K_{ATP} Channel Sulfonylurea Sensitivity by a cAMP Analog Selective for the cAMP-Regulated Guanine Nucleotide Exchange Factor Epac"

Kelley, G.G.; Chepurny, O.G.; Schwede, F.; Genieser, H.-G.; Leech, C.A.; Roe, M.W.; Li, X.; Dzhura, I.; Dzhura, E.; Afshari, P.; Holz, G.G., *Islets*, **1**, 260 - 265 (2009): "Glucose-dependent Potentiation of Mouse Islet Insulin Secretion by Epac Activator 8-pCPT-2'-O-Me-cAMP-AM"

Chepurny, O.G.; Leech, C.A.; Kelley, G.G.; Dzhura, I.; Dzhura, E.; Li, X.; Rindler, M.J.; Schwede, F.; Genieser, H.-G.; Holz, G.G., *J. Biol. Chem.*, **284**, 10728 - 10736 (2009): "Enhanced Rap1 Activation and Insulin Secretagogue Properties of an Acetoxymethyl Ester of an Epac-selective cyclic AMP Analog in Rat INS-1 Cells: Studies with 8-pCPT-2'-O-Me-cAMP-AM"

Vliem, M.J.; Ponsioen, B.; Schwede, F.; Pannekoek, W.-J.; Riedl, J.; Kooistra, M.R.H.; Jalink, K.; Genieser, H.-G.; Bos, J.L.; Rehmann, H., *ChemBioChem.*, **9**, 2052 - 2054 (2008): "8-pCPT-2'-O-Me-cAMP-AM: An Improved Epac-selective cAMP Analogue"

Selected References for 8-pCPT-2'-O-Me-cAMP:

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"

Christensen, A.E.; Doeskeland, S.O., *Handbook of Cell Signaling*, Vol. 2, Academic Press/Elsevier Science, San Diego, CA, 549 - 554 (2003): "Cyclic Nucleotide Analogs as Tools to Investigate Cyclic Nucleotide Signaling"

Bos, J.L., *Nature Rev. Mol. Cell Biol.*, **4**, 733 - 738 (2003): "Epac: A New cAMP Target and New Avenues in cAMP Research"

Rehmann, H.; Schwede, F.; Doeskeland, S.O.; Wittinghofer, A.; Bos, J.L., *J. Biol. Chem.*, **278**, 38548 - 38556 (2003): "Ligand-mediated Activation of the cAMP-responsive Guanine Nucleotide Exchange Factor Epac"

Christensen, A.E.; Selheim, F.; de Rooij, J.; Dremier, S.; Schwede, F.; Dao, K.K.; Martinez, A.; Maenhaut, C.; Bos, J.L.; Genieser, H.-G.; Doeskeland, S.O., *J. Biol. Chem.*, **278**, 35394-35402 (2003): "cAMP Analog Mapping of Epac1 and cAMP-Kinase. Discriminating Analogs Demonstrate that Epac and cAMP-Kinase Act Synergistically to Promote PC-12 Cell Neurite Extension"

Eliasson, L.; Ma, X.S.; Renström, E.; Barg, S.; Berggren, P.O.; Galvanovskis, J.; Gromada, J.; Jing, X.J.; Lundquist, I.; Salehi, A.; Sewing, S.; Rorsman, P., *J. Gen. Physiol.*, **121**, 181 - 197 (2003): "SUR1 Regulates PKA-independent cAMP-induced Granule Priming in Mouse Pancreatic B-cells"

Rangarajan, S.; Enserink J.M.; Kuiperij, H.B.; de Rooij, J.; Price, L.S.; Schwede, F.; Bos, J.L., *J. Cell Biol.*, **160**, 487 - 493 (2003): "Cyclic AMP Induces Integrin-mediated Cell Adhesion Through Epac and Rap1 Upon Stimulation of the β_2 -Adrenergic Receptor"

Kang, G.; Joseph, J.W.; Chepurny, O.G.; Monaco, M.; Wheeler, M.B.; Bos, J.L.; Schwede, F.; Genieser, H.-G.; Holz, G.G., *J. Biol. Chem.*, **278**, 8279 - 8285 (2003): "Epac-selective cAMP Analog 8-pCPT-2'-O-Me-cAMP as a Stimulus for Ca^{2+} -induced Ca^{2+} Release and Exocytosis in Pancreatic β -Cells"

Enserink J.M.; Christensen, A.E.; de Rooij, J.; van Triest, M.; Schwede, F.; Genieser, H.-G.; Doeskeland, S.O.; Blank, J.L.; Bos, J.L., *Nature Cell Biol.*, **4**, 901 - 906 (2002): "A novel Epac-specific cAMP Analog Demonstrates Independent Regulation of Rap1 and ERK"

Selected Reference for AM-ester:

Kruppa, J., Keely, S., Schwede, F., Schultz, C., Barrett, K.E.; Jastorff, B., *Bioorg. Med. Chem. Lett.* **7**, 945 - 948 (1997): "Bioactivatable Derivatives of 8-Substituted cAMP- Analogues"