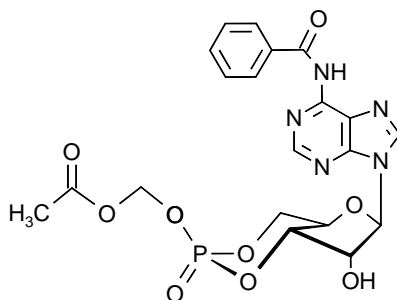


## Technical Information about the Acetoxymethyl Ester of N<sup>6</sup>-Benzoyl-cAMP (6-Bnz-cAMP-AM)

Membrane-permeant, metabolically activatable activator of cAMP-dependent protein kinases, but poor Epac agonist

Update: June 08, 2011 WH



### Abbreviation:

**6-Bnz-cAMP-AM**

Formula	CAS No.	Molecular Weight	UV	BIOLOG Cat.No.
C <sub>20</sub> H <sub>20</sub> N <sub>5</sub> O <sub>9</sub> P	[pending]	505.4	λ <sub>max</sub> 279 nm / ε 17000 / pH 7	B 079

**Name:** N<sup>6</sup>- Benzoyladenosine- 3', 5'- cyclic monophosphate, acetoxymethyl ester

**Description:** 6-Bnz-cAMP-AM is an analogue of the protein kinase A activator N<sup>6</sup>-Benzoyl-cAMP (6-Bnz-cAMP, Cat. No. B 009) in which the polar phosphate is masked by an acetoxymethyl group.

**Properties:** The acetoxymethyl group of 6-Bnz-cAMP-AM masks the charged polar phosphate and thus makes the molecule highly membrane-permeant. Inside the cell esterases release the more polar 6-Bnz-cAMP which is a potent, site-selective activator of cAMP-dependent protein kinase. 6-Bnz-cAMP does not activate Epac and thus can be used as an Epac-negative control. Phosphate tris(acetoxymethyl)ester, PO<sub>4</sub>-AM<sub>3</sub> (Cat. No. P 030), is recommended as control reagent in 6-Bnz-cAMP-AM applications to test for side effects of enzymatically released acetic acid and formaldehyde, two metabolites with potential biological functions.

**Specification:** Lyophilized or crystallized solid. Please note that equal concentrations of 6-Bnz-cAMP-AM can appear very different in volume due to sensitivity of the lyophilized form to humidity. Micromolar quantities are determined by UV at λ<sub>max</sub>.

**Purity:** Typical analysis is better than 97% (HPLC / UV / 279 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 6-Bnz-cAMP-AM in water or buffers is limited. We suggest to use a small amount of anhydrous organic solvent such as anhydrous DMSO or DMF for dissolution at 1-100 mM, and to dilute with water or buffer down to the concentrations required. In some cases, especially at high concentrations (~ 1mM), Pluronic® F-127 (Molecular Probes) can be useful to facilitate solubilization in physiological media. Please keep in mind that due to the high potency of 6-Bnz-cAMP-AM relatively low concentrations (0.005-0.1 mM) should be sufficient, and be sure to check for DMSO/DMF tolerance in your system. Since 6-Bnz-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:** 6-Bnz-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 6-Bnz-cAMP-AM:**

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"

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 Ca<sup>2+</sup> by Glucagon-like Peptide-1 Receptor Agonist Exendin-4 is Disrupted in Beta-Cells of Phospholipase C-Epsilon Knockout Mice"

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<sub>ATP</sub> Channel Sulfonylurea Sensitivity by a cAMP Analog Selective for the cAMP-Regulated Guanine Nucleotide Exchange Factor Epac"

**Selected Reference for 6-Bnz-cAMP:**

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.; Døskeland, 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"

**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"