

Product Data Sheet

Product Name: PKG Substrate

Cat. No.: GC34227

Chemical Properties

Cas. No.

SMILES Arg-Lys-Arg-Ser-Arg-Ala-Glu

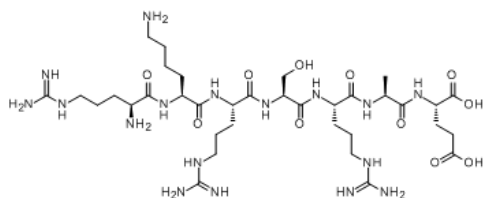
Formula $C_{35}H_{67}N_{17}O_{11}$ M.Wt 902.01

Solubility Soluble in Water Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Caution: Product has not been fully validated for medical applications. For research use only.

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Kinase experiment:

Kinase activity is measured by determining the amount of ^{33}P radioactivity incorporated from ^{33}P ATP or ^{33}P N6-benzyl-ATP into a PKG specific peptide substrate (RKRSRAE). The standard 75 μL assay mixture contains 0.15 μCi of ^{33}P ATP, 10 μM ATP, 15 μM PKG peptide substrate, 2 μM PKI (a synthetic peptide inhibitor of cAMP-dependent protein kinase), 1 μg of purified kinase, and 100 μM 8-Br-cGMP in 50 mM HEPES buffer, pH 7.4, containing 10 mM MgCl_2 , 0.1% Tween 20, and 1 mM DTT. After incubation at 30°C for 2 min, the reaction is immediately put on ice, and 20 μL of the assay mixture is spotted onto P81 phosphocellulose paper and then quenched in 0.42% H_3PO_4 . The paper is further washed three times in 0.42% H_3PO_4 for 10 min with gentle agitation and rinsed once with acetone. After air drying, radioactivity on the paper is measured with a Beckman LS6500 liquid scintillation counter. For measuring the effect of N6-benzyl-ATP on the activity of PKG I utilizing ATP as a co-substrate, unlabeled N6-benzyl-ATP is added to each reaction at the indicated concentrations. Saturation kinetic analyses for K_m and V_{max} with ATP or N6-benzyl-ATP are performed over a concentration range (0.0015-100 μM) by adding unlabeled ATP or N6-benzyl-ATP to a given amount of ^{33}P ATP or ^{33}P N6-benzyl-ATP, respectively[1].

References:

[1]. Wong A, et al. Cyclic GMP-dependent stimulation of serotonin transport does not involve direct transporter phosphorylation by cGMP-dependent protein kinase. J Biol Chem. 2012 Oct 19;287(43):36051-8.

Background

PKG Substrate is a selective substrate for cGMP-dependent protein kinase (PKG).

Incorporation of ^{33}P ATP into the synthetic peptide PKG substrate RKRSRAE is measured. N6-benzyl-ATP inhibits kinase activity of PKG $\text{I}\alpha$ gatekeeper mutants but not WT. The serotonin transporter (SERT) is responsible for reuptake of serotonin (5-hydroxytryptamine) after its exocytotic release from neurons. SERT is regulated by several processes, including a cyclic GMP signaling

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pathway involving nitric oxide synthase, guanylyl cyclase, and PKG[1].

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