PKG Substrate

Cat. No.: HY-P1561

CAS No.: 81187-14-6

Molecular Formula: $C_{35}H_{67}N_{17}O_{11}$

Molecular Weight: 902.01

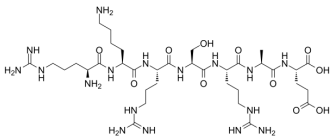
Sequence: Arg-Lys-Arg-Ser-Arg-Ala-Glu

Sequence Shortening: RKRSRAE

Target: Others

Pathway: Others

Storage: Sealed storage, away from moisture and light, under nitrogen
Powder -80°C 2 years
-20°C 1 year
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light, under nitrogen)



SOLVENT & SOLUBILITY

In Vitro

H_2O : 100 mg/mL (110.86 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.1086 mL	5.5432 mL	11.0864 mL
	5 mM	0.2217 mL	1.1086 mL	2.2173 mL
	10 mM	0.1109 mL	0.5543 mL	1.1086 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: PBS
Solubility: 100 mg/mL (110.86 mM); Clear solution; Need ultrasonic

BIOLOGICAL ACTIVITY

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

IC_{50} & Target PKG[1]

In Vitro Incorporation of [^{33}P]ATP into the synthetic peptide PKG substrate RKRSRAE is measured. N^6-benzyl-ATP inhibits kinase activity of PKG Iα 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 pathway involving nitric oxide synthase, guanylyl cyclase, and PKG[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]

Kinase activity is measured by determining the amount of ^{33}P radioactivity incorporated from [^{33}P]ATP or [^{33}P]N^6-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 N^6-benzyl-ATP on the activity of PKG I utilizing ATP as a co-substrate, unlabeled N^6-benzyl-ATP is added to each reaction at the indicated concentrations. Saturation kinetic analyses for K_m and V_{max} with ATP or N^6-benzyl-ATP are performed over a concentration range (0.0015-100 µM) by adding unlabeled ATP or N^6-benzyl-ATP to a given amount of [^{33}P]ATP or [^{33}P]N^6-benzyl-ATP, respectively[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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.

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

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