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 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ 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	PKG Substrate		

Protocol

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

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Kinase experiment:

Kinase activity is measured by determining the amount of 33P radioactivity incorporated from [33P]ATP or [33P]N6-benzyl-ATP into a PKG specific peptide substrate (RKRSRAE). The standard 75 µL assay mixture contains 0.15 µCi of [33P]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 MgCl2, 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% H3PO4. The paper is further washed three times in 0.42% H3PO4 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 Km and Vmax 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 [33P]ATP or [33P]N6-benzyl-ATP, respectively[1].

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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 [33P]ATP into the synthetic peptide PKG substrate RKRSRAE is measured. N6-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].

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