

PDE3A, Active

Human recombinant protein expressed in Sf9 cells

Catalog # P91-31G

Lot # X562-3

Product Description

Recombinant human PDE3A (669-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_000921](#).

Gene Aliases

CGI-PDE

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Scientific Background

PDE3A is a member of the phosphodiesterase family of proteins that play a critical role in regulating intracellular levels of cAMP and cGMP. PDE3A has high affinity for both cAMP and cGMP and shows competitive inhibition of the cAMP hydrolytic activity by cGMP (1). Deletion of the N-terminus of PDE3A enhances the hydrolysis of cGMP relative to cAMP suggesting that the role of divergent N-termini of various PDEs could be to exert substrate specificity. Stimulation of platelets with thrombin increases PDE3A activity via Akt signaling pathway and this increase can be diminished by dephosphorylation of PDE3A by protein phosphatase 1 (2).

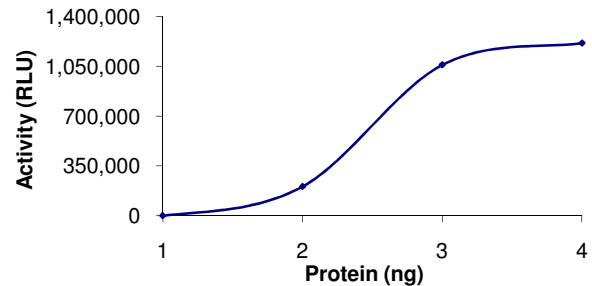
References

- Meacci E, et al: Molecular cloning and expression of human myocardial cGMP-inhibited cAMP phosphodiesterase. Proc. Nat. Acad. Sci. 89: 3721-3725, 1992.
- Zhang W, et al: Thrombin regulates intracellular cyclic AMP concentration in human platelets through phosphorylation/activation of phosphodiesterase 3A. Blood. 2007 Sep 1;110(5):1475-82.

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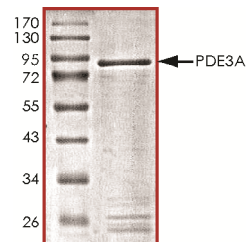
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Specific Activity



The specific activity of PDE3A was determined to be **550 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PDE3A was determined to be **>90%** by densitometry. Approx. MW **84kDa**.

PDE3A, Active

Human recombinant protein expressed in Sf9 cells

Catalog Number	P91-31G
Specific Activity	550 nmol/min/mg
Specific Lot Number	X562-3

Purity	>90%
Concentration	0.1µg/µl
Stability	1yr At -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

Activity Assay Protocol

Reaction Components

Active PDE3A (Catalog #: P91-31G)

Active PDE3A (0.1µg/µl) diluted with 1X PDE-Glo™ Reaction Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PDE3A for optimal results).

100 mM IBMX Solution

Prepare 100 mM of 3-isobutyl-1-methylxanthine (IBMX) in 100% DMSO. Store aliquots at -20°C.

PDE-Glo™ Phosphodiesterase Assay Kit (Promega, Cat # V1361)

cAMP and cGMP solution, 1 mM
PDE-Glo™ Reaction Buffer, 5X
PDE-Glo™ Termination Buffer, 5X
PDE-Glo™ Detection Buffer, 5X
Protein Kinase A (PKA)
Kinase-Glo™ Substrate
Kinase-Glo™ Buffer

Assay Protocol

The PDE3A assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE3A reaction between an active PDE3A preparation and a cyclic nucleotide substrate (cAMP). Then PDE-Glo™ Termination Buffer and PDE-Glo™ Detection Buffer (which contains ATP, inactive PKA and PKA substrate) are added to the reaction. The cyclic nucleotide substrate remaining after the PDE3A reaction can bind to the inactive PKA regulatory subunit thereby releasing the active catalytic subunit of PKA. The active catalytic subunit of PKA then catalyzes phosphorylation of the PKA substrate in the presence of ATP which leads to a reduction in ATP level. In the final step, Kinase-Glo™ reagent is added to measure the Luciferase activity towards Luciferin and the luminescent signal produced is related to the amount of ATP remaining which is indirectly related to the activity of PDE3A.

Step 1. Thaw the Active PDE3A and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- Diluted active PDE3A with 1X PDE-Glo™ Reaction Buffer on ice
- 2µM cAMP substrate solution in 1X PDE-Glo™ Reaction Buffer at ambient temperature
- 1X PDE-Glo™ Termination Buffer in 10 mM IBMX solution at ambient temperature
- 1X PDE-Glo™ detection solution (mix 8µl PKA with 792µl water and 200µl 5X PDE-Glo™ Detection Buffer). Prepare immediately before use
- Kinase-Glo™ reagent by adding Kinase-Glo™ Buffer to Kinase-Glo™ Substrate at ambient temperature

Step 3. In a polystyrene 96-well plate, add the following components bringing the initial reaction volume up to 25µl:

Component 1. 12.5µl of diluted Active PDE3A (Catalog #P91-31G)

Component 2. 12.5µl of 2µM cAMP solution (0.025 nmol cAMP used per assay)

Step 4. Set up a blank control as outlined in step 3 by excluding the addition of the diluted PDE preparation. Replace the PDE preparation with an equal volume of 1X PDE-Glo™ Reaction Buffer.

Step 5. Initiate the reaction by adding cAMP substrate solution and incubate the mixture at 30°C for 10 minutes on a plate shaker.

Step 6. Terminate the PDE reaction by adding 12.5µl of 1X PDE-Glo™ Termination Buffer. Mix well.

Step 7. Add 12.5µl of 1X PDE-Glo™ detection solution. Mix well and then incubate at ambient temperature for 20 minutes.

Step 8. After the incubation period, add 50µl of Kinase-Glo™ reagent mix and then incubate at ambient temperature for 10 min.

Step 9. Read the polystyrene 96-well reaction plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

Step 10. Perform a cAMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmol cAMP remaining after the PDE reaction from the standard curve.

Step 11. Calculate the PDE specific activity as outlined below.

PDE Specific Activity (SA) (nmol/min/mg)

$$[\text{cAMP total (nmol)} - \text{cAMP remaining (nmol)}] / (\text{Reaction time in min}) * (\text{Enzyme amount in mg})$$

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