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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P90-30G -05	5 µg
P90-30G -10	10 µg
P90-30G -20	20 µg

PDE2A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P90-30G
Lot # H067-3

Product Description

Recombinant full-length human PDE2A was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002599.

Gene Aliases

PDE2A1; PED2A4

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 50mM NaCl, 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.

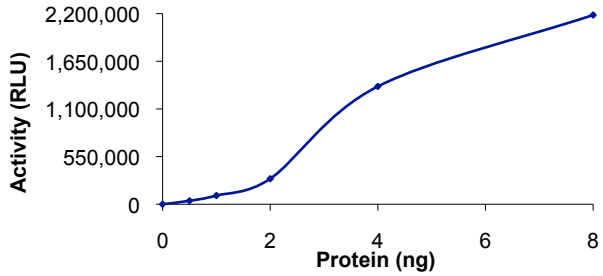
Scientific Background

PDE2A is a member of the cyclic nucleotide phosphodiesterase family. PDE2A is a cGMP-specific phosphodiesterase and hydrolyzes cGMP to 5'-GMP. TNFα stimulation of cells leads to an increase in PDE2A expression in a p38 mitogen-activated protein kinase (MAPK)-dependent manner (1). TNFα-mediated up-regulation of PDE2A may destabilize endothelial barrier function in sepsis. In addition, selective PDE2A up-regulation sensitizesendothelial cells toward the permeability-increasing agent thrombin (2). Furthermore, PDE2A inhibition is effective in preventing thrombin-induced lung edema.

References

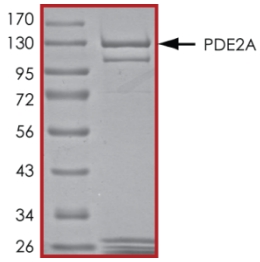
1. Seybold J, et al: Tumor necrosis factor-alpha-dependent expression of phosphodiesterase 2: role in endothelial hyperpermeability. Blood. 2005 May 1;105(9):3569-76.
2. Diebold I, et al: Phosphodiesterase 2 mediates redox-sensitive endothelial cell proliferation and angiogenesis by thrombin via Rac1 and NADPH oxidase 2. Circ Res. 2009 May 22;104(10):1169-77.

Specific Activity



The specific activity of PDE2A was determined to be **5750 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>70%** by densitometry.
Approx. MW **130kDa**.

PDE2A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P90-30G
Specific Activity	5750 nmol/min/mg
Specific Lot Number	H067-3
Purity	>70%
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.

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Activity Assay Protocol

Reaction Components

Active PDE2A (Catalog #: P90-30G)

Active PDE2A (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 PDE2A 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 PDE2A assay is performed using the PDE-Glo™Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE2A reaction between an active PDE2A preparation and cyclic nucleotide substrate cGMP. 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 PDE2A 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 PDE2A.

Step 1. Thaw the Active PDE2A and PDE-Glo™Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- Diluted active PDE2A with 1X PDE-Glo™ Reaction Buffer on ice
- 20μM cGMP substrate solution in1X 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 200μl5X PDE-Glo™ Detection Buffer). Prepare immediately before use
- Kinase-Glo™ reagent by addingKinase-Glo™ Buffer to Kinase-Glo™ Substrateat 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 PDE2A (Catalog #P90-30G)

Component 2. 12.5μl of 20μM cGMP solution (0.25 nmol cGMP 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 reactionby adding cGMP 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 cGMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmol cGMP 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)

[cGMP total (nmol)- cGMP remaining (nmol)] / (Reaction time in min)*(Enzyme amount in mg)

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