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Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P94-30BG -05	5 µg
P94-30BG -10	10 µg

PDE6B, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P94-30BG
Lot # S334-2

Product Description

Full-length recombinant human PDE6B was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_000283.

Gene Aliases

rd1; PDEB; RP40; CSNB3

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.

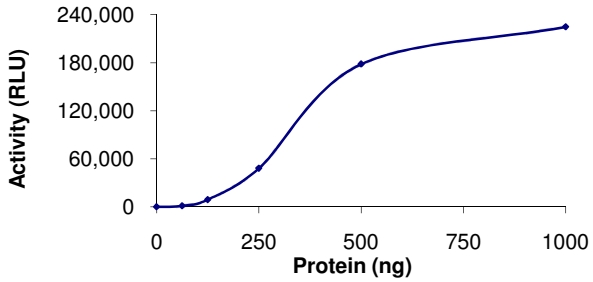
Scientific Background

PDE6B is a member of the phosphodiesterase family of proteins that play a critical role in regulating intracellular levels of cAMP and cGMP. PDE6B is a high-affinity cGMP-specific PDE that shows high expression in the eye. Four mutations in the PDE6B gene lead to a degenerative disease of photoreceptors called retinitis pigmentosa and autosomal dominant congenital stationary night blindness (1). Subretinal injection of mice with retinal degeneration with PDE6B gene showed significant decrease in photoreceptor cell death (2). A nonsense mutation in the PDE6B gene is also the cause of Rod-cone dysplasia-1 (rcd1) in Irish setters.

References

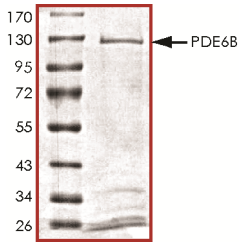
1. McLaughlin M E, et al: Recessive mutations in the gene encoding the beta-subunit of rod phosphodiesterase in patients with retinitis pigmentosa. Nat Genet. 1993 Jun;4(2):130-4.
2. Bennett J, et al: Photoreceptor cell rescue in retinal degeneration (rd) mice by in vivo gene therapy. Nature *Med.* 2: 649, 1996.

Specific Activity



The specific activity of PDE6B was determined to be **20 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PDE6B was determined to be **>70%** by densitometry. Approx. MW **124kDa**.

PDE6B, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number	P94-30BG
Specific Activity	20nmol/min/mg
Specific Lot Number	S334-2
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 PDE6B (Catalog #: P94-30BG)

Active PDE6B (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 PDE6B 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 PDE6B assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE6B reaction between an active PDE6B 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 PDE6B 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 PDE6B.

Step 1. Thaw the Active PDE6B and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- Diluted active PDE6B with 1X PDE-Glo™ Reaction Buffer on ice
- 20µM cGMP 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 PDE6B (Catalog #P94-30BG)

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 reaction by 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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