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Product Information

PDE6B, active, GST tagged, human recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5059**
Storage Temperature –70 °C

Synonyms: rd1; PDEB; RP40; CSNB3

Product Description
PDE6B is a member of the phosphodiesterase family of proteins that plays 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.

Full-length recombinant human PDE6B was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_000283. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~124 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 16–22 nmole/min/mg (see Figure 2)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

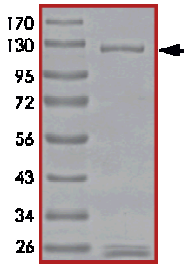
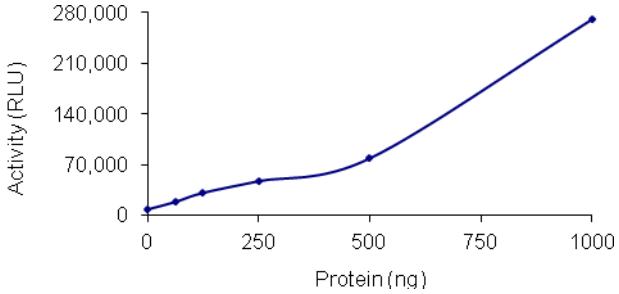


Figure 2.
Specific Activity of Typical Lot
16–22 nmole/min/mg



Procedure
Preparation Instructions
PDE-Glo™ Phsophodiesterase Assay Kit (Promega, Cat No. V1361)

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

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

Phosphodiesterase Solution – Dilute the active PDE6B (0.1 μg/μl) with 1× PDE-Glo Reaction Buffer to the desired concentration.
Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PDE6B for optimal results.

Phosphodiesterase Assay
The PDE6B assay is performed using the PDE-Glo Phosphodiesterase Assay kit (Promega; Cat. No. V1361). The assay involves first a PDE6B reaction between an active PDE6B preparation and a 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.

1. Thaw the active PDE6B and PDE-Glo assay kit reagents on ice.
2. Prepare the following working solutions:
   - Diluted active PDE6B with 1× PDE-Glo Reaction Buffer on ice
   - 20 μM cGMP substrate solution in 1× PDE-Glo Reaction Buffer at room temperature
   - 1× PDE-Glo Termination Buffer in 10 mM IBMX solution at room temperature
   - 1× PDE-Glo Termination Buffer in 10 mM IBMX solution at room temperature
   - 1× PDE-Glo detection solution (mix 8 μl of PKA with 792 μl of water and 200 μl of 5× PDE-Glo Detection Buffer). Prepare immediately before use
   - Kinase-Glo reagent by adding Kinase-Glo Buffer to Kinase-Glo Substrate at room temperature
3. In a polystyrene 96-well plate, add the following solutions to a volume of 25 μl:
   12.5 μl of diluted active PDE6B
   12.5 μl of 20 μM cGMP solution (0.25 nmole cGMP used per assay)
   Note: Do not add cGMP until step 5
4. Set up a blank control as outlined in step 3, excluding the addition of the diluted PDE preparation. Replace the PDE preparation with an equal volume of 1× PDE-Glo Reaction Buffer.
5. Initiate each reaction with the addition of 12.5 μl of 20 μM cGMP Solution, bringing the final reaction volume to 25 μl. Incubate the mixture at 30 °C for 10 minutes on a plate shaker.
6. Terminate the PDE reaction by adding 12.5 μl of PDE-Glo Termination Buffer. Mix well.
7. Add 12.5 μl of 1× PDE-Glo detection solution. Mix well and then incubate at room temperature for 20 minutes.
8. After the incubation period, add 50 μl of Kinase-Glo reagent mix and then incubate at room temperature for 10 minutes.
9. Read the 96-well reaction plate using the Kinase-Glo Luminescence Protocol on a GloMax® plate reader (Promega, Cat No. E7031).
10. Create a cGMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmole cGFMP remaining after the PDE reaction from the standard curve.
11. Calculate the PDE specific activity.

Calculations:
1. PDE Specific Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta[\text{cGMP}]}{E \times T}$$

Δ[cGMP] = cGMP total concentration in nmole minus cGMP concentration remaining
T = reaction time (minutes)
E = amount of enzyme (mg)

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., **4**, 130-134 (1993).
2. Bennett J., et al., Photoreceptor cell rescue in retinal degeneration (rd) mice by *in vivo* gene therapy. Nature Med., **2**, 649 (1996).