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Product Information

PDE6A (31-end), active, GST tagged, human recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5058**
Storage Temperature –70 °C

Synonym: CGPR-A

Product Description
PDE6A encodes the cyclic-GMP (cGMP)-specific phosphodiesterase 6A and is expressed in cells of the retinal rod outer segment. The phosphodiesterase 6 holoenzyme is a heterotrimer composed of an alpha, beta, and two gamma subunits. cGMP is an important regulator of rod cell membrane current, and its dynamic concentration is established by phosphodiesterase 6A cGMP hydrolysis and guanylate cyclase cGMP synthesis.[1] The protein is a subunit of a key phototransduction enzyme and participates in processes of transmission and amplification of the visual signal. Mutations in this gene have been identified as one cause of autosomal recessive retinitis pigmentosa.[2]

Recombinant human PDE6A (31-end) was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_000440. 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: ~120 kDa

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

Specific Activity: 32–44 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)

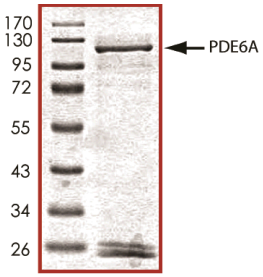
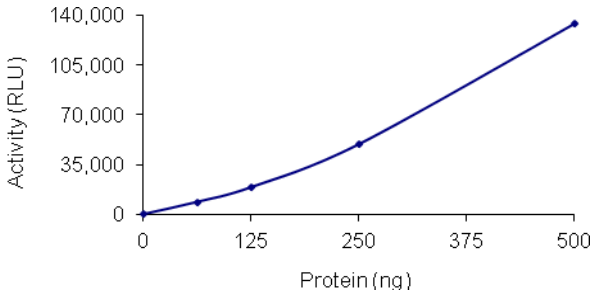


Figure 2.
Specific Activity of Typical Lot
32–44 nmole/min/mg



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 PDE6A (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 PDE6A for optimal results.

Phosphodiesterase Assay
The PDE6A assay is performed using the PDE-Glo Phosphodiesterase Assay kit (Promega; Cat. No. V1361). The assay involves first a PDE6A reaction between an active PDE6A 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 PDE6A 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 PDE6A.

1. Thaw the active PDE6A and PDE-Glo assay kit reagents on ice.
2. Prepare the following working solutions:
   - Diluted active PDE6A 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 PDE6A
   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. Pittler, S.J., et al., Molecular characterization of human and bovine rod photoreceptor cGMP phosphodiesterase alpha-subunit and chromosomal localization of the human gene. Genomics, **6**, 272-283 (1990).
2. Danciger, M., et.al., Genetic mapping demonstrates that the alpha-subunit of retinal cGMP-phosphodiesterase is not the site of the rd mutation. Exp. Eye Res., **51**, 185-189 (1990).

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