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Product Information

PDE8B (113-end), active, GST tagged, human recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5060**
Storage Temperature –70 °C

Synonyms: ADSD; FLJ11212; PPNAD3

Product Description
PDE8B or phosphodiesterase 8B is a cyclic nucleotide phosphodiesterase (PDE) that catalyzes the hydrolysis of the second messenger cAMP.[1] The recombinant PDE8B exhibits cAMP-PDE activity that is resistant to several PDE inhibitors. The cAMP hydrolytic activity of PDE8B is unaffected by cGMP and it does not display any cGMP mediated hydrolysis. Defects in PDE8B are a cause of autosomal dominant striatal degeneration (ADSD).[2] PDE8B plays a prominent role in degrading cAMP expression in human embryonic kidney cells.

Recombinant human PDE8B (113-end) was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_003719. 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: ~115 kDa

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

Specific Activity: 34–46 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)

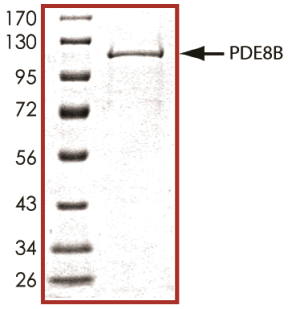
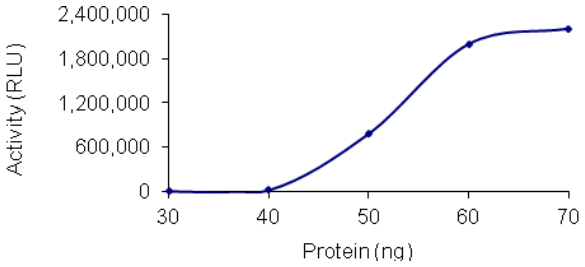


Figure 2.
Specific Activity of Typical Lot
34–46 nmole/min/mg



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 PDE8B (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 PDE8B for optimal results.

Phosphodiesterase Assay
The PDE8B assay is performed using the PDE-Glo Phosphodiesterase Assay kit (Promega; Cat. No. V1361). The assay involves first a PDE8B reaction between an active PDE8B 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 PDE8B 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 PDE8B.

1. Thaw the active PDE8B and PDE-Glo assay kit reagents on ice.
2. Prepare the following working solutions:
   - Diluted active PDE8B with 1× PDE-Glo Reaction Buffer on ice
   - 2 μM cAMP 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 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 PDE8B
   12.5 μl of 2 μM cAMP solution (0.025 nmole cAMP used per assay)
   Note: Do not add cAMP 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 2 μM cAMP 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 cAMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmole cAMP remaining after the PDE reaction from the standard curve.
11. Calculate the PDE specific activity.

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

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

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

References

1. Hayashi, M., et al., Genomic organization, chromosomal localization, and alternative splicing of the human phosphodiesterase 8B gene. Biochem. Biophys. Res. Comm., **297**, 1253-1258 (2002).
2. Horvath, A., et al., A cAMP-specific phosphodiesterase (PDE8B) that is mutated in adrenal hyperplasia is expressed widely in human and mouse tissues: a novel PDE8B isoform in human adrenal cortex. Europ. J. Hum. Genet., **16**, 1245-1253 (2008).

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