

PDE8A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog # P96-30G

Lot # F496-1

Product Description

Full-length recombinant human PDE8A was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_002605](#).

Gene Aliases

FLJ16150; HsT19550

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.25mM DTT, 0.1mM PMSF, and 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

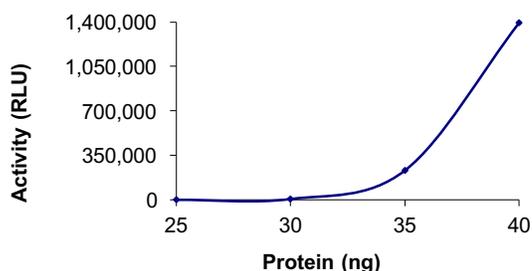
PDE8A is a member of the phosphodiesterase family of proteins that play a critical role in regulating intracellular levels of cAMP and cGMP. PDE8A is a high-affinity cAMP-specific PDE expressed in a variety of tissues such as ovary and testis. PDE8A plays an important role in many biological processes, including T-cell activation, testosterone production, adrenocortical hyperplasia, and thyroid function. Targeted mutation in the PDE8A gene in Leydig cells show sensitivity in the action of LH in terms of testosterone production (1). PDE8A, through its PAS domain, can bind with IκB proteins in a region containing their ankyrin repeats. The association of PDE8A with IκB greatly enhanced the enzyme activity this enzyme (2).

References

- Chen C, et al: PDE8A genetic variation, polycystic ovary syndrome and androgen levels in women. *Mol Hum Reprod.* 2009 Aug;15(8):459-69.
- Wu P, et al: Per-Arnt-Sim domain-dependent association of cAMP-phosphodiesterase 8A1 with IκB proteins. *Proc Natl Acad Sci U S A.* 2004 Dec 21;101(51):17634-9.

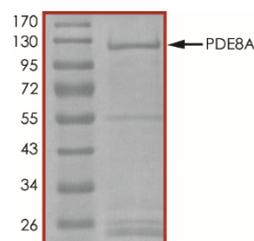
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Specific Activity



The specific activity of PDE8A was determined to be **91.8 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>80%** by densitometry.
Approx. MW **120kDa**.

PDE8A, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog #	P96-30G
Specific Activity	91.8 nmol/min/mg
Lot #	F496-1
Purity	>80%
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.

Activity Assay Protocol

Reaction Components

Active PDE8A (Catalog #: P96-30G)

Active PDE8A (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 PDE8A 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 PDE8A assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE8A reaction between an active PDE8A 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 PDE8A 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 PDE8A.

Step 1. Thaw the Active PDE8A and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- Diluted active PDE8A with 1X PDE-Glo™ Reaction Buffer on ice
- 2µM cAMP 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 PDE8A (Catalog #P96-30G)

Component 2. 12.5µl of 2µM cAMP solution (0.025 nmol cAMP 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 cAMP 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 cAMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmol cAMP 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)

$$[\text{cAMP total (nmol)} - \text{cAMP remaining (nmol)}] / (\text{Reaction time in min}) * (\text{Enzyme amount in mg})$$

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