

PDE5A, Active

Human recombinant protein expressed in Sf9 cells

Catalog # P93-31G

Lot # I340-3

Product Description

Recombinant human PDE5A (537-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM_001083](#).

Gene Aliases

CN5A; PDE5; PDE5A1; CGB-PDE

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

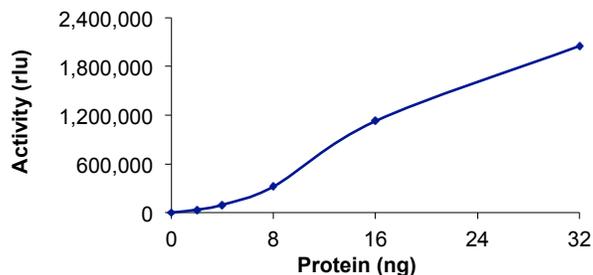
PDE5A is a member of the cyclic nucleotide phosphodiesterase family. PDE5A is a cGMP-specific phosphodiesterase and hydrolyzes cGMP to 5'-GMP. It is involved in the regulation of intracellular concentrations of cyclic nucleotides and is important for smooth muscle relaxation in the cardiovascular system. PDE5A regulates nitric oxide (NO)-induced relaxation and cGMP breakdown in smooth muscle cells in vas deferens (1). PDE5A expression predisposes mice to adverse left ventricular remodeling after myocardial infarction. Furthermore, increased myocardial PDE5A expression in patients with advanced cardiomyopathy can contribute to the development of heart failure (2).

References

- Mancina R, et al: Expression and functional activity of phosphodiesterase type 5 in human and rabbit vas deferens. *Mol Hum Reprod*. 2005 Feb;11(2):107-15.
- Pokreisz P, et al: Ventricular phosphodiesterase-5 expression is increased in patients with advanced heart failure and contributes to adverse ventricular remodeling after myocardial infarction in mice. *Circulation*. 2009 Jan 27;119(3):408-16.

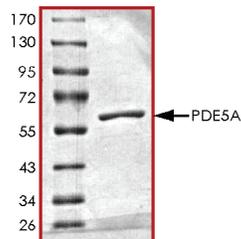
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Specific Activity



The specific activity of PDE5A was determined to be **1940 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **63kDa**.

PDE5A, Active

Human recombinant protein expressed in Sf9 cells

Catalog #	P93-31G
Specific Activity	1940 nmol/min/mg
Lot #	I340-3
Purity	>90%
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 PDE5A (Catalog #: P93-31G)

Active PDE5A (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 PDE5A 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 PDE5A assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE5A reaction between an active PDE5A 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 PDE5A 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 PDE5A.

Step 1. Thaw the Active PDE5A and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- o Diluted active PDE5A with 1X PDE-Glo™ Reaction Buffer on ice
- o 20µM cGMP substrate solution in 1X PDE-Glo™ Reaction Buffer at ambient temperature
- o 1X PDE-Glo™ Termination Buffer in 10 mM IBMX solution at ambient temperature
- o 1X PDE-Glo™ detection solution (mix 8µl PKA with 792µl water and 200µl 5X PDE-Glo™ Detection Buffer). Prepare immediately before use
- o 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 PDE5A (Catalog #P93-31G)

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)

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

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