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1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P92-31DG -05	5 µg
P92-31DG -10	10 µg
P92-31DG -20	20 µg

PDE4D, Active

Human recombinant protein expressed in Sf9 cells

Catalog # P92-31DG
Lot # R052-1

Product Description

Recombinant human PDE4D (225-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_006203.

Gene Aliases

DPDE3; STRK1; HSPDE4D; PDE4DN2

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 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.

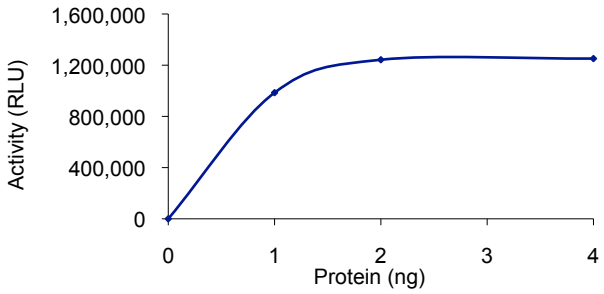
Scientific Background

PDE4D is a member of the phosphodiesterase (PDE) family that is cAMP-specific and has homology to the 'dunce' gene of Drosophila. PDE4D is expressed in both cytosolic and particulate fractions in the cell and Rolipram, a specific PDE4 inhibitor, inhibits and significantly lowers the IC50 for the cytosolic forms of the enzyme. TNFα upregulates the basal expression of PDE4D in HUVEC. PDE4D, which is not detected in untreated cells, accumulates beginning 4 hours after TNFα treatment and increased by 24 hours (1). Mice deficient in PDE4D exhibit delayed growth as well as reduced viability and female fertility (2).

References

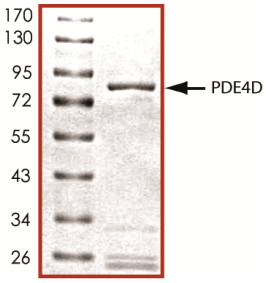
1. Miro X, et al: Phosphodiesterases 4D and 7A splice variants in the response of HUVEC cells to TNF-alpha. *Biochem. Biophys. Res. Commun.* 274: 415-421, 2000.
2. Jin S-L C, et al: Impaired growth and fertility of cAMP-specific phosphodiesterase PDE4D-deficient mice. *Proc. Nat. Acad. Sci.* 96: 11998-12003, 1999.

Specific Activity



The specific activity of PDE4D was determined to be **1200 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>80%** by densitometry.
Approx. MW **82kDa**.

PDE4D, Active

Human recombinant protein expressed in Sf9 cells

Catalog Number	P92-31DG
Specific Activity	1200 nmol/min/mg
Specific Lot Number	R052-1
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.

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Activity Assay Protocol

Reaction Components

Active PDE4D (Catalog #: P92-31DG)

Active PDE4D (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 PDE4D 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 PDE4D assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE4D reaction between an active PDE4D 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 PDE4D 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 PDE4D.

Step 1. Thaw the Active PDE4D and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- Diluted active PDE4D 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 PDE4D (Catalog #P92-31DG)

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)

[cAMP total (nmol) - cAMP remaining (nmol)] / (Reaction time in min)*(Enzyme amount in mg)

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