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Specialists in Signaling Proteins
1-866-9 KINASE (54-6273)

Catalogue #	Aliquot Size
P95-31G -05	5 µg
P95-31G -10	10 µg
P95-31G -20	20 µg

PDE7A, Active

Human recombinant protein expressed in Sf9 cells

Catalog # P95-31G
Lot # M333-2

Product Description

Recombinant human PDE7A (104-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_002603.

Gene Aliases

HCP1; PDE7

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.

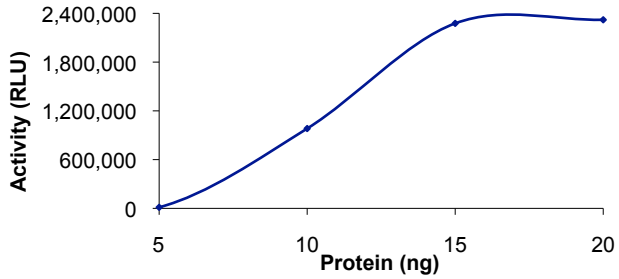
Scientific Background

PDE7A is a member of the phosphodiesterase family of proteins that play a critical role in regulating intracellular levels of cAMP and cGMP. PDE7A is a high-affinity cAMP-specific PDE and is expressed in T cell lines, peripheral blood T lymphocytes, epithelial cell lines, airway and vascular smooth muscle cells, lung fibroblasts, and eosinophils. PDE7 plays a critical role in the regulation of human T cell function and selective PDE7 inhibitors are being examined to treat immunological and inflammatory disorders (1). PDE7A plays an important role in the regulation of osteoblastic differentiation (2). PDE7A depletion by RNAi upregulates expression of several osteogenic genes and increases mineralization.

References

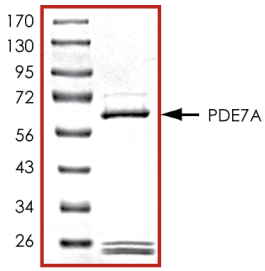
1. Nakata A, et al: Potential role of phosphodiesterase 7 in human T cell function: comparative effects of two phosphodiesterase inhibitors. Clin Exp Immunol. 2002 Jun;128(3):460-6.
2. Pekkinen M, et al: Effects of phosphodiesterase 7 inhibition by RNA interference on the gene expression and differentiation of human mesenchymal stem cell-derived osteoblasts. Bone. 2008 Jul;43(1):84-91.

Specific Activity



The specific activity of PDE7A was determined to be **166 nmol /min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>85%** by densitometry.
Approx. MW **64kDa**.

PDE7A, Active

Human recombinant protein expressed in Sf9 cells

Catalog Number	P95-31G
Specific Activity	166 nmol/min/mg
Specific Lot Number	M333-2
Purity	>85%
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 PDE7A (Catalog #: P95-31G)

Active PDE7A (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 PDE7A 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 PDE7A assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE7A reaction between an active PDE7A 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 PDE7A 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 PDE7A.

Step 1. Thaw the Active PDE7A and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

Step 2. Prepare the following working solutions:

- Diluted active PDE7A 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 PDE7A (Catalog #P95-31G)

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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