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1-866-9 KINASE (54-6273)

| Catalog # | Aliquot Size |
|---|---|
| P89-30BG-05 | 5 µg |
| P89-30BG-10 | 10 µg |

# PDE1B, Active

Full-length recombinant protein expressed in Sf9 cells

**Catalog # P89-30BG**

*Lot # B2215-7*

## Product Description

Full-length recombinant human PDE1B was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_000924.

## Gene Aliases

PDE1B1; PDES1B

## Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 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.

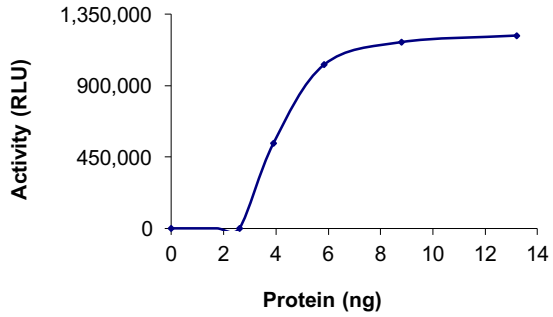
## Scientific Background

PDE1B is a member of the PDE protein superfamily that catalyzes the hydrolysis of the cyclic nucleotides cAMP and cGMP to the corresponding nucleoside 5-prime-monophosphates. PDE1B is a calmodulin-dependent enzyme that is stimulated by calcium-calmodulin complex (1). PDE1B is express in the lymphoblastic and leukemic cell lines and causes the cells to undergo apoptosis, suggesting PDE1B could be a novel therapeutic strategy for the treatment of leukemia (2). GM-CSF is a major regulator of monocyte to macrophage differentiation and this cytokine has been shown to up-regulate PDE1B.

## References

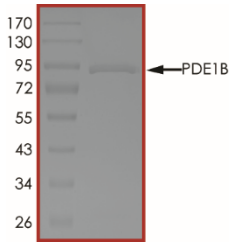

1. Yu, J.et.al: Identification and characterisation of a human calmodulin-stimulated phosphodiesterase PDE1B1. Cell. Signal. 9: 519-529, 1997.
2. Jiang, X. et.al: Inhibition of calmodulin-dependent phosphodiesterase induces apoptosis in human leukemic cells. Proc. Nat. Acad. Sci. 93: 11236-11241, 1996.


## Specific Activity




The specific activity of PDE1B was determined to be **100 nmol /min/mg** as per activity assay protocol.

## Purity




The purity of PDE1B was determined to be **>90%** by densitometry. Approx. MW **86kDa**.


# PDE1B, Active

Full-length recombinant protein expressed in Sf9 cells


| | |
|---|---|
| Catalog # | P89-30BG |
| Specific Activity | 100 nmol/min/mg |
| Lot # | B2215-7 |
| 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. |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# Activity Assay Protocol

## Reaction Components

**Active PDE1B** (Catalog #: P89-30BG)

Active PDE1B (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 PDE1B 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 PDE1B assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE1B reaction between an active PDE1B 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 PDE1B 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 PDE1B.

**Step 1.** Thaw the Active PDE1B and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

**Step 2.** Prepare the following working solutions:

- Diluted active PDE1B 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.** 10µl of diluted Active PDE1B (Catalog #P89-30BG)

**Component 2.** 2.5µl of $Ca^{2+}$/Calmodulin solution (10X) (Catalog #C02-39)

**Component 3.** 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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