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Product Information

PDE1C, active, GST tagged, human recombinant, expressed in *Sf*9 cells

Catalog Number **SRP5057**
Storage Temperature –70°C

Synonym: Hcam3

Product Description

PDE1C is a member of the phosphodiesterases (PDE) family, which catalyzes the hydrolysis of cyclic nucleotides cAMP and cGMP to the corresponding nucleoside 5′-monophosphates. PDE1C1 binds both cAMP and cGMP with high affinity, and hydrolyzes both substrates with similar rates of catalysis.[1] PDE1C is a calmodulin-dependent PDE and is stimulated by calcium-calmodulin complex. PDE1C is expressed at high levels in human cardiac myocytes with an intracellular distribution distinct from that of other phosphodiesterases.[2] PDE1C levels decrease in all conditions that inhibited cell proliferation, and PDE1C is a useful marker in studying the dynamics of proliferation and migration of various cells.

Full-length recombinant human PDE1C was expressed by baculovirus in *Sf*9 insect cells using an N-terminal GST tag. The gene accession number is NM_005020. Recombinant protein stored in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM glutathione, 0.1 mM EDTA, 0.25 mM DTT, 0.1 mM PMSF, and 25% glycerol.

Molecular mass: ~99 kDa

Purity: 70–95% (SDS-PAGE, see Figure 1)

Specific Activity: 378–512 nmole/min/mg (see Figure 2)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product ships on dry ice and storage at –70 °C is recommended. After opening, aliquot into smaller quantities and store at –70 °C. Avoid repeated handling and multiple freeze/thaw cycles.

Figure 1.
SDS-PAGE Gel of Typical Lot
70–95% (densitometry)

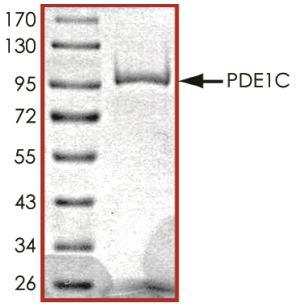
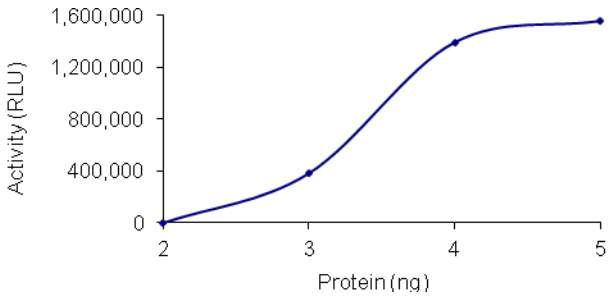


Figure 2.
Specific Activity of Typical Lot
378–512 nmole/min/mg



Procedure

Preparation Instructions

PDE-Glo™ Phsophodiesterase Assay Kit (Promega, Cat No. V1361)

- cAMP and cGMP solution, 1 mM
- PDE-Glo Reaction Buffer, 5×
- PDE-Glo Termination Buffer, 5×
- PDE-Glo Detection Buffer, 5×
- Protein Kinase A (PKA)
- Kinase-Glo® Substrate
- Kinase-Glo Buffer

100 mM IBMX Solution - Prepare 100 mM of 3-isobutyl-1-methylxathine (IBMX) in 100% DMSO. Store aliquots at –20 °C.

Phosphodiesterase Solution – Dilute the active PDE1C (0.1 µg/µl) with 1× PDE-Glo Reaction Buffer to the desired concentration.
Note: The specific activity plot may be used as a guideline (see Figure 2). It is recommended the researcher perform a serial dilution of active PDE1C for optimal results.

Phosphodiesterase Assay
The PDE1C assay is performed using the PDE-Glo Phosphodiesterase Assay kit (Promega; Cat. No. V1361). The assay involves first a PDE1C reaction between an active PDE1C 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 PDE1C 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 PDE1C.

1. Thaw the active PDE1C and PDE-Glo assay kit reagents on ice.
2. Prepare the following working solutions:
   - Diluted active PDE1C with 1× PDE-Glo Reaction Buffer on ice
   - 2 µM cAMP substrate solution in 1× PDE-Glo Reaction Buffer at room temperature
   - 1× PDE-Glo Termination Buffer in 10 mM IBMX solution at room temperature
   - 1× PDE-Glo Termination Buffer in 10 mM IBMX solution at room temperature
   - 1× PDE-Glo detection solution (mix 8 µl of PKA with 792 µl of water and 200 µl of 5× PDE-Glo Detection Buffer). Prepare immediately before use
   - Kinase-Glo reagent by adding Kinase-Glo Buffer to Kinase-Glo Substrate at room temperature
3. In a polystyrene 96-well plate, add the following solutions to a volume of 25 µl:
   10 µl of diluted active PDE1C
   2.5 µl of Ca^{2+}/Calmodulin solution (10×)
   12.5 µl of 2 µM cAMP solution (0.025 nmole cAMP used per assay)
   Note: Do not add cAMP until step 5
4. Set up a blank control as outlined in step 3, excluding the addition of the diluted PDE preparation. Replace the PDE preparation with an equal volume of 1× PDE-Glo Reaction Buffer.
5. Initiate each reaction with the addition of 12.5 µl of 2 µM cAMP Solution, bringing the final reaction volume to 25 µl. Incubate the mixture at 30 °C for 10 minutes on a plate shaker.
6. Terminate the PDE reaction by adding 12.5 µl of PDE-Glo Termination Buffer. Mix well
7. Add 12.5 µl of 1× PDE-Glo detection solution. Mix well and then incubate at room temperature for 20 minutes.
8. After the incubation period, add 50 µl of Kinase-Glo reagent mix and then incubate at room temperature for 10 minutes.
9. Read the 96-well reaction plate using the Kinase-Glo Luminescence Protocol on a GloMax® plate reader (Promega, Cat No. E7031).
10. Create a cAMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmole cAMP remaining after the PDE reaction from the standard curve.
11. Calculate the PDE specific activity.

Calculations:
1. PDE Specific Activity (SA) (nmole/min/mg)

$$\text{nmole/min/mg} = \frac{\Delta[\text{cAMP}]}{E \times T}$$

Δ[cAMP] = cAMP total concentration in nmole minus cAMP concentration remaining
T = reaction time (minutes)
E = amount of enzyme (mg)

References

1. Loughney, K. et al., Isolation and characterization of cDNAs corresponding to two human calcium, calmodulin-regulated, 3-prime,5-prime-cyclic nucleotide phosphodiesterases. J. Biol. Chem., **271**, 796-806 (1996).
2. Vandeput, F. et al., Cyclic nucleotide phospho-diesterase PDE1C1 in human cardiac myocytes. J. Biol. Chem., **282**, 32749-42757 (2007).

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