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## Data Sheet

### PDE4D2

human, recombinant, N-terminal GST tag  
**Catalog #:** 60043

Formulated in: 25 mM Tris-HCl, pH 8.0,  
100 mM NaCl, 0.05% Tween-20, 50% glycerol,  
and 3 mM DTT.

Stability: >6 months at -80°C

References:

1. Fidani,L., et al., Eur. J. Neurol. 14 (7), 745-749 (2007).
2. Baillie,G.S., et al., Biochem. J. 404 (1), 71-80 (2007).

Description:

Human PDE4D2 (GenBank Accession No. NM\_006203), full length, with N-terminal GST tag, MW=103 kDa, expressed in a Baculovirus infected Sf9 cell expression system.

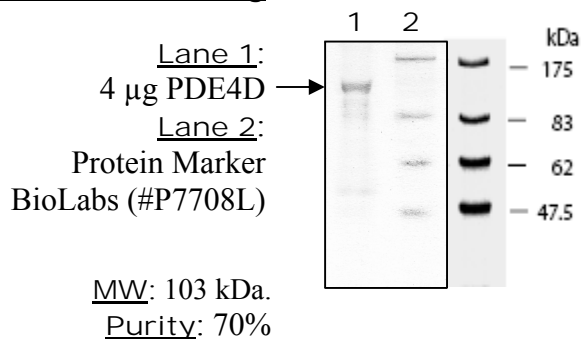
Specific Activity: 36365 pmol/min/μg. 1 unit is defined as the amount of enzyme that will convert 1 pmole of 3', 5'-cAMP to 5' AMP per min at 37 °C in a reaction buffer. Assay conditions: 10 mM Tris-HCl, pH7.4, 10 mM MgCl<sub>2</sub>, 1 mM MnCl<sub>2</sub>, 200 μM cAMP, 2.5 kU 5' nucleotidase, 37°C, 20 min.

Application:

Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

### Quality Assurance

10% SDS-PAGE  
Coomassie staining



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## **Assay Protocol**

**Materials:** IMAP<sup>TM</sup> TR-FRET Screening Express with Progressive Binding Kit from Molecular Devices (R8160); 2'-Fluo-AHC-cAMP from Axxora LLC (BLG-F003-01, San Diego, CA); PDE4D2 (BPS Catalog Number 60043).

### **Methods:**

#### **Step 1:**

Dilute FAM-cAMP to 200nM in 1X PDE Assay Buffer.  
Dilute PDE4D2 enzymes to 0.015ng/ $\mu$ l in 1X PDE Assay Buffer.

#### **Step 2:**

Adding following components to a low binding black plate:  
25 $\mu$ l of 200nM FAM-cAMP (Final concentration will be 100nM)  
5 $\mu$ l of 1 xPDE assay buffer  
20 $\mu$ l of PDE4D2 (0.015ng/ $\mu$ l) (Final amount=0.3ng/reaction)  
Mix and incubate at room temperature for 1 hour.

#### **Step 3:**

Prepare 1X reagent-binding buffer (85% 1X Binding Buffer A and 15% 1X Binding Buffer B).  
Prepare Binding Solution by diluting Binding Reagent with 1X reagent-binding Buffer (1:600).  
Add 120 $\mu$ l of Binding Solution to each well and incubate the plate at room temperature for 1 hour.

#### **Step 4:**

Measure fluorescence polarization at excitation of 485nm and emission of 520nm in BioTek Synergy<sup>TM</sup> 2 microplate reader.

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