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Data Sheet

PDE4B1

Human, recombinant, N-terminal GST tag
Catalog #: 60041

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. Shah, V.O., et al., Am. J. Kidney Dis. 48 (4), 616-628 (2006)
2. Smith, P.G., et al., Blood 105 (1), 308-316 (2005).

Description:

Human PDE4B1 (GenBank Accession No. NM_001037341), full length with N-terminal GST tag, MW= 109 kDa, expressed in a Baculovirus infected Sf9 cell expression system.

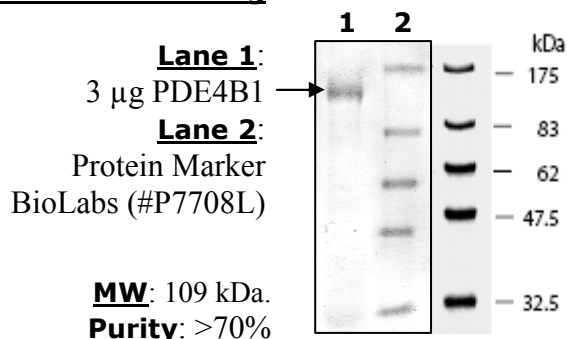
Specific Activity: 10000 U/μg. 1 unit is defined as the amount of enzyme that will convert 1 pmole of 3', 5'-cAMP to 5' AMP per min at 30 °C in a reaction buffer of 10 mM Tris-HCl, pH 7.4, 0.2 mM MgCl₂. Assay buffer: 10 mM Tris-HCl, pH 7.4, 1 mM MgCl₂, 0.4 mM 3', 5'-cAMP substrate, and 0.2 ng/μl PDE4B1.

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™ TR-FRET Screening Express with Progressive Binding Kit from Molecular Devices (R8160); FAM-Cyclic-3',5'-AMP from Molecular Devices (R7505); PDE4B1 (BPS Catalog Number 60041).

Methods:

Step 1:

Dilute FAM-cAMP to 200nM in 1X PDE Assay Buffer.
Dilute PDE4B1 enzymes to 0.06 ng/μl in 1X PDE Assay Buffer.

Step 2:

Adding following components to a low binding black plate:
25μl of 200nM FAM-cAMP (Final concentration will be 100nM)
5 μl of 1 x PDE assay buffer
20μl of PDE4B1 (0.06ng/μl) (Final amount=1.2ng/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 120μl 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™ 2 microplate reader.

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