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Data Sheet

PDE9A2

Human, recombinant, N-terminal GST tag
Catalog #: 60090

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. Rentero C. and Puigdomenech P. BMC Mol. Biol. 7, 39 (2006).
2. Huai Q. et al., Journal Proc. Natl. Acad. Sci. U.S.A. 101 (26), 9624-9629 (2004).

Description:
Human PDE9A2 (GenBank Accession No. NM_001001567), full length with N-terminal GST tag, MW=87.7 kDa, expressed in a Baculovirus infected Sf9 cell expression system.

Specific Activity: 97 pmol/min/µg. Assay conditions: 10 mM Tris-HCl, pH7.4, 10 mM MgCl2, 1mM MnCl2, 400 µM cGMP, 2.5 kU 5' nucleotidase, 5 ng/µl PDE9A, 37ºC, 20min.

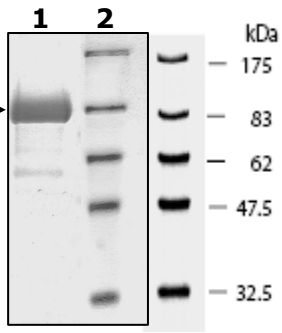
Application:
Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

Quality Assurance

10% SDS-PAGE
Coomassie staining

Lane 1:
4 µg PDE9A2
Lane 2:
Protein Marker
BioLabs (#P7708L)

MW: 87.7 kDa.
Purity: >80%



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Assay Protocol

Materials: IMAP™ TR-FRET Screening Express with Progressive Binding Kit from Molecular Devices (R8160); FAM-Cyclic-3',5'-GMP purchased from AXXORA (San Diego, CA).; PDE9A2 (BPS Catalog Number 60090).

Methods:

Step 1:
Dilute FAM-cGMP to 200nM in 1X PDE Assay Buffer.
Dilute PDE9A2 enzymes to 0.0125ng/µl in 1X PDE Assay Buffer.

Step 2:
Adding following components to a low binding black plate:
25µl of 200nM FAM-cGMP (Final concentration will be 100nM)
5µl of PDE assay buffer
20µl of PDE9A2 (0.0125ng/µl) (Final amount=0.25ng/reaction)
Mix and incubate at room temperature for 1 hour.

Step 3:
Prepare 1X reagent-binding buffer (75% 1X Binding Buffer A and 25% 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.