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Data Sheet

PDE7A/CRE Reporter - HEK293 Cell Line
Catalog #: 60413

Description

Phosphodiesterases (PDEs) regulate the intracellular levels of cAMP and cGMP by hydrolyzing cAMP and cGMP to their inactive 5´ monophosphates. These cyclic nucleotides play an important role as second messengers in diverse physiological functions. PDE7 is a cAMP-specific enzyme and two PDE7 genes (PDE7A and PDE7B) have been identified. PDE7A is widely expressed in various tissues including skeletal muscle, T lymphocytes, brain and pancreas. Inhibition of PDE7 activity by its inhibitors leads to elevated intracellular level of cAMP.

The regulation of intracellular level of cAMP by PDE7A in cells can be monitored by a specific reporter for the cAMP pathway, the CRE luciferase reporter. CRE luciferase reporter contains a luciferase gene that is under the control of the cAMP response element (CRE). Elevation of intracellular cAMP activates cAMP response element binding protein (CREB) to bind CRE and induces the expression of luciferase.

The PDE7A/CRE Reporter-HEK293 line contains the firefly luciferase gene under the control of CRE as well as a constitutive expression construct for human PDE7A (phosphodiesterase 7A, accession number NM_002603), both stably integrated into HEK293 cells. The luciferase expression level from the CRE reporter is used to monitor the activity of PDE7A in the cells. The cell line is validated for the induction of the expression of luciferase reporter by an inhibitor of PDE7A.

Application

- Monitor human PDE7A activity.
- Screen for PDE7A inhibitors.

Format

Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO.

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Functional Validation and Assay Performance

N-terminal FLAG-tagged human PDE7A is stably expressed in a human embryonic kidney (HEK293) cell line. PDE7A expression was confirmed by Western blotting.

PDE7A activity was monitored by CRE luciferase activity assay. Forskolin is commonly used to raise the intracellular level of cAMP in cell physiology studies. Forskolin resensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing cAMP levels. When cells were activated by forskolin, the level of cAMP was upregulated in control HEK293 cells containing only CRE luciferase reporter (CRE Reporter-HEK293) inducing high expression of the CRE luciferase reporter. However, PDE7A/CRE Reporter-HEK293 cells showed reduction in the level of forskolin-induced cAMP, resulting in lowered expression of luciferase. Inhibition of PDE7A activity by BRL 50481, a PDE7A inhibitor, restored the cAMP level, resulting in higher luciferase activity (See figure 1., below).

Assay performance: Induction of CRE reporter activity by PDE7A inhibitor in PDE7A /CRE Reporter – HEK293 cells

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volumes should be scaled appropriately.

Materials Required but Not Supplied

- BRL50481 (Enzo # BML-PD120): PDE7A inhibitor. Prepare 100 mM of stock solution in DMSO.
- Forskolin: prepare 10 mM of stock solution in DMSO.
- Assay medium: growth medium without Geneticin and Hygromycin
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate (Corning # 3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690) or other luciferase reagent for measuring firefly luciferase activity
- Luminometer

1. Harvest cells from culture in Growth medium and seed cells at a density of ~30,000 cells per well in 40µl of assay medium in a white clear-bottom 96-well microplate.

2. Dilute PDE7A inhibitor (BRL50481) stock in assay medium. Add 10 µl of diluted inhibitor to wells. The final DMSO concentration in our assay is 0.1% (the final DMSO concentration may be up to 0.5%).

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Add 10 µl of assay medium containing the same concentration of DMSO to control wells without inhibitor.
Add 50 µl of assay medium with 0.1% DMSO to cell-free control wells (for determining background luminescence).

Tap the plate gently to mix. Make sure that the cells distribute evenly in the wells.

3. Incubate at 37°C in a CO_2 incubator for ~ 16 hours.

4. The next day, cells should be ~ 80% confluent. Dilute Forskolin in assay medium to 10 µM and add 5.5 µl of diluted Forskolin to stimulated wells. The final Forskoin concentration in the wells is 1 µM.

   Add 5.5 µl of assay medium with the same concentration of DMSO to the unstimulated control wells and cell-free control wells.

   Tap the plate *very gently* to mix. Make sure that cells remain attached to the wells.

   Set up each treatment in at least triplicate.

5. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

6. Perform luciferase assay using ONE-Step™ luciferase assay system: Add 50 µl of ONE-Step Luciferase reagent per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer. If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.

7. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.
   The fold induction of CRE luciferase reporter expression = average background-subtracted luminescence of Forskolin-stimulated wells / average background-subtracted luminescence of unstimulated control wells

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Figure 1. PDE7A overexpression reduces the level of cAMP following forskolin stimulation in PDE7A /CRE-HEK293 cells. This effect is reversed by BRL 50481, a PDE7A inhibitor.
The data are shown as fold induction of CRE luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without forskolin treatment (fold induction =background-subtracted luminescence of Forskolin-stimulated wells / average background-subtracted luminescence of unstimulated control wells).

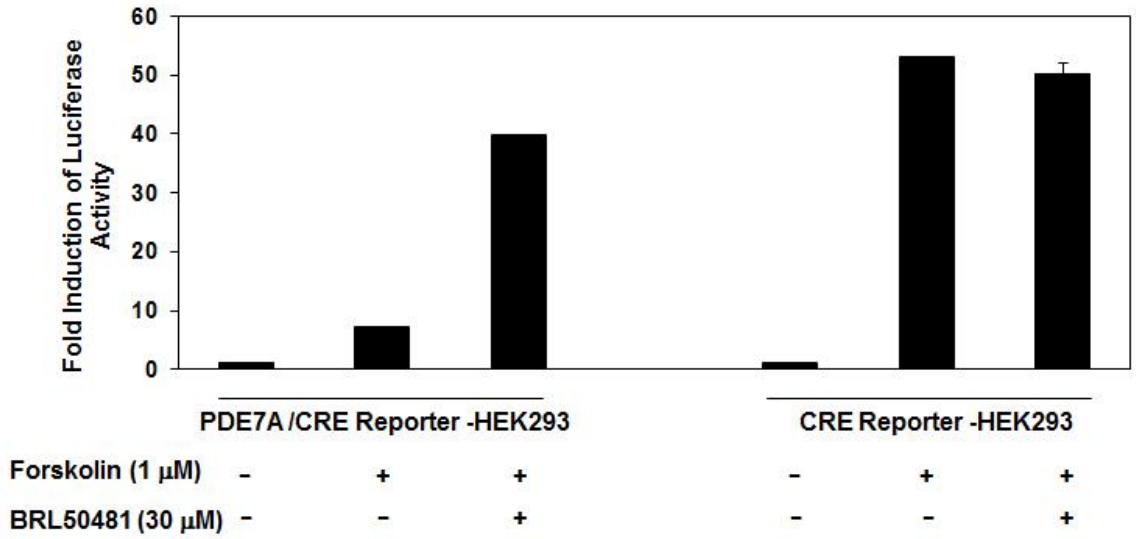

Figure 2. BRL50481 inhibition dose response curve in PDE7A /CRE Reporter cells.

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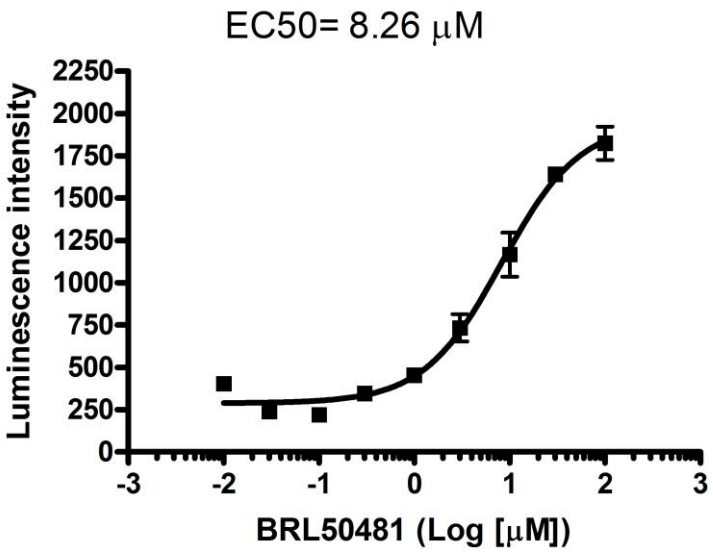
The result is shown as background-subtracted luminescence intensity. The inhibition of PDE7A in cells induces luminescence, so the inhibitory effects of BRL50481 on PDE7A activity is expressed as EC50. The EC50 of BRL50481 is ~ 8.26 µM.



Mycoplasma testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

Storage
Store cells in liquid nitrogen upon arrival.

Culture conditions
Cells should be grown at 37°C with 5% CO_2 using MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) medium supplemented with 10% FBS (Life technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01), plus 400 µg/ml of Geneticin (Life technologies #11811031) and 50 µg/ml of Hygromycin B (Hyclone #SV30070.01). It may be necessary to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium **without**

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Geneticin and Hygromycin, spin down cells, and resuspend cells in pre-warmed growth medium **without** Geneticin and Hygromycin. Transfer resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C overnight. The next day, replace the medium with fresh growth medium **without** Geneticin and Hygromycin, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. At first passage, switch to growth medium containing Geneticin and Hygromycin. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from the culture vessel with 0.05% Trypsin/EDTA. Add complete growth medium and transfer to a tube, spin down the cells, then resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly or twice a week.

Vector and sequence
N-terminal FLAG-tagged human PDE7A (accession number NM_002603) was cloned into pcDNA3.1 vector (Invitrogen).

Polylinker: CMV-HindIII-KpnI-BamHI-**PDE7A**-XhoI-XbaI-ApaI------SV40-neomycin^R

hPDE7A sequence:
MDYKDDDDKGITLIWCLALVLIKWITSKRRGAISYDSSDQTALYIRMLGDVRVRS
RAGFESERRGSHPYIDFRIFHSQSEIEVSVSARNIRRLLSFQRYLRSSRFFRGTA
VSNSLNILDDDYNGQAKCMLEKVGNWNFDIFLFDRLTNGNSLVSLTFHLFSLHG
LIEYFHLDMMKLRRFLVMIQEDYHSQNPYHNAVHAADVTQAMHCYLKEPKLAN
SVTPWDILLSLIAAATHDLDHPGVNQPFLIKTNHYLATLYKNTSVLENHHWRSAV
GLLRESGLFSHLPLESRQQMETQIGALILATDISRQNEYLSLFRSHLDRGDLCLE
DTRHRHLVLQMALKCADICNPCRTWELSKQWSEKVTEEFFHQGDIEKKYHLGV
SPLCDRHTESIANIQIGFMTYLVEPLFTEWARFSNTRLSQTMLGHVGLNKASWK
GLQREQSSSEDTDAAFELNSQLLPQENRLS

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References

1. Malik, R. *et al.* (2008) Cloning, stable expression of human phosphodiesterase 7A and development of an assay for screening of PDE7 selective inhibitors. *Appl. Microbiol. Biotechnol.* **77 (5):** 1167-1173
2. Fan Chung, K. (2006) Phosphodiesterase inhibitors in airways disease. *Eur. J. Pharmacol.* **533(1-3):**110-117

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Related Products

Product Name	Catalog #	Size
CRE Reporter-HEK293 cell line	60613	2 vials
PDE7A-HEK293 Cell line	60407	2 vials
PDE7B-HEK293 Cell line	60412	2 vials
Rat PDE7A-HEK293 Cell line	60408	2 vials
CRE/CREB Kit (cAMP/PKA)	60611	500 rxns.
PDE4D Cell-Based Activity Assay Kit	60505	500 rxns.
PDE7A Enzyme (Human)	60070	10 µg
PDE7B Enzyme (Human)	60071	10 µg
PDE7A Enzyme (Rat)	60074	10 µg
PDE7B Enzyme (Rat)	60075	10 µg