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Data Sheet

PDE1B Cell-Based Reporter Assay Kit

Catalog #: 60601

Background

PDE1B plays a key role in signal transduction by regulating the intracellular concentration of cyclic nucleotides. PDE1B hydrolyzes both cAMP and cGMP to nucleoside 5′-monophosphate and prefers cGMP at low substrate levels.

Description

The PDE1B Cell-Based Reporter Assay Kit is designed for screening inhibitors of PDE1B in cultured cells. The kit is also ideal for monitoring PDE1B/ cGMP/ Ca2+ channel signaling pathway activity in vivo. The kit includes a recombinant HEK293 cell line that stably expresses human PDE1B, atrial natriuretic peptide (ANP), mammalian expression constructs for ANP receptor A, CNGA2, and aequorin, and buffer for the assay.

In the PDE1B reporter assay, PDE1B-HEK293 cells are transiently transfected with expression vectors for atrial natriuretic peptide (ANP) receptor A (ANPRA, a guanylate cyclase), cyclic nucleotide-gated cation channel CNGA2, and the photoprotein aequorin. When cells are activated with ANP, ANP induces the production of cGMP through ANPRA. cGMP can activate the CNGA2 channel and induces Ca^{2+} influx, resulting in aequorin luminescence. Therefore, the intracellular cGMP level is monitored via aequorin luminescence induced by Ca^{2+} influx through CNGA2, acting as the intracellular cGMP sensor. PDE1B can reduce the ANP-induced cGMP level in the cells, causing lowered aequorin luminescence. When PDE1B activity is inhibited (for example, by the PDE1 inhibitor BAY607550), then the ANP-induced cGMP level increases, resulting in calcium influx that stimulates luminescence signals. A schematic presentation of this assay is shown in Figure 1.

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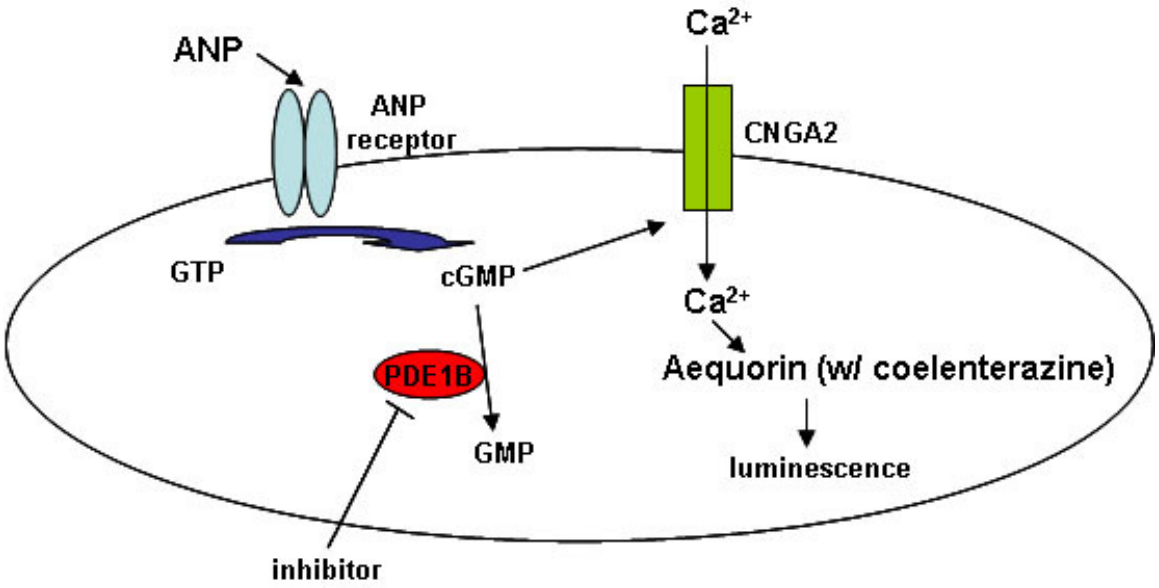
Figure 1. Schematic presentation of cGMP assay used to characterize the function of PDE1B.



Components

Component	Cat. #	Amount	Storage
PDE1B-HEK293 cell line	60402	1 vial	liquid nitrogen
ANPRA/aequorin Expression vector*	60641	20 µg (80 ng DNA/ µl)	-20°C
CNGA2 Expression vector*	60642	20 µg (80 ng DNA/ µl)	-20°C
Atrial natriuretic peptide (ANP), 10 µM	60349	50 µl	-20°C
Coelenterazine, 5 mM	60395	50 µl	-20°C
Ca^{2+} free assay buffer	60396	100 mL	+4°C
BSA (100X)	60397	500 µl	-20°C
$CaCl_2$, 1M	60398	200 µl	+4°C

**These vectors are suitable for transient transfection. They are NOT suitable for transformation and amplification in bacteria.*

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Materials Required but Not Supplied

- Complete cell growth medium **(**MEM/EBSS supplemented with 10% FBS, 1% non-essential amino acid, 1 mM Na-pyruvate, 1% Penn-Strep and 400 µg/ml Geneticin)
- 96-well Poly-D-Lysine coated white clear-bottom assay plate
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen # 11668027). However, other transfection reagents work equally well.]
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- Luminometer

Cell Culture Conditions

PDE1B-HEK293 Cells should be grown at 37° with 7% CO_2 using MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na-pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01), and 400 µg/ml of Geneticin (Invitrogen #11811031). PDE1B-HEK293 cells should exhibit a typical cell division time of 24 hours. *Note: If culturing cells in medium from other vendors, it may be required to lower 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 Geneticin, spin down cells, resuspend cells in pre-warmed growth medium without Geneticin, transfer resuspended cells to T25 flask and culture in a 37° CO_2 incubator. The next day, change the medium to growth medium containing Geneticin.

Cells should be split before they reach complete confluency. To passage the cells, pre-wash cells with phosphate-buffered saline (PBS), detach cells from culture vessel with Trypsin/EDTA (Hyclone #SH30236.01), add complete growth medium and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly.

Assay Protocol

All amounts and volumes in the following protocol are provided on a per well basis.

1. Harvest PDE1B-HEK293 cells and seed cells at a density of ~30,000 cells per well into Poly-D-Lysine coated white clear-bottom 96-well microplate in 100 µl of growth medium without Geneticin. Incubate cells at 37°C and 7% CO_2 overnight to allow them to recover and reattach. Cells will be ~90% confluent at the time of transfection.

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2. The next day, transiently transfect the cells with ANP receptor, CNGA2, and aequorin. For each well, prepare complexes as follows:
   a. Dilute 0.5 µl of CNGA2 expression vector and 0.5 µl of ANPRA/aequorin expression vector in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently.

   b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature.

   c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

   d. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate. Incubate cells at 37° in a CO_2 incubator for 2 days.

Note: we recommend setting up the assay in at least quadricate for each treatment. To minimize pipetting errors, prepare a master mix of sufficient transfection cocktail for multiple wells.

3. Approximately 48 hours after transfection, perform the cGMP reporter assay as follows:
   a. Prepare coelenterazine solution: dilute coelenterazine 1000-fold in Ca^{2+} free assay buffer.

   b. Dilute PDE1B inhibitor or DMSO in coelenterazine solution. The final [DMSO] should not exceed 0.3%.

   c. Remove cell medium and replace with 100 µl of coelenterazine solution containing PDE1B inhibitor or DMSO. Incubate cells at 37° in a CO_2 incubator for 3 hours.

   d. Prepare ANP solution by diluting the BSA stock 100-fold in Ca^{2+} free assay buffer, then diluting the ANP 1000-fold into this BSA-containing Ca^{2+} free assay buffer. Dilute the PDE1B inhibitor (or control DMSO) into the ANP solution at same concentration as step b. Prepare this solution right before adding to cells.

   e. Three hours after incubation, remove the coelenterazine solution from cells and replace with 90 µl of ANP solution containing PDE1B inhibitor or DMSO. Include wells without ANP treatment as a negative control, and add the buffers to wells without any cells to measure any background luminescence. Incubate for ~15 min and measure aequorin luminescence.

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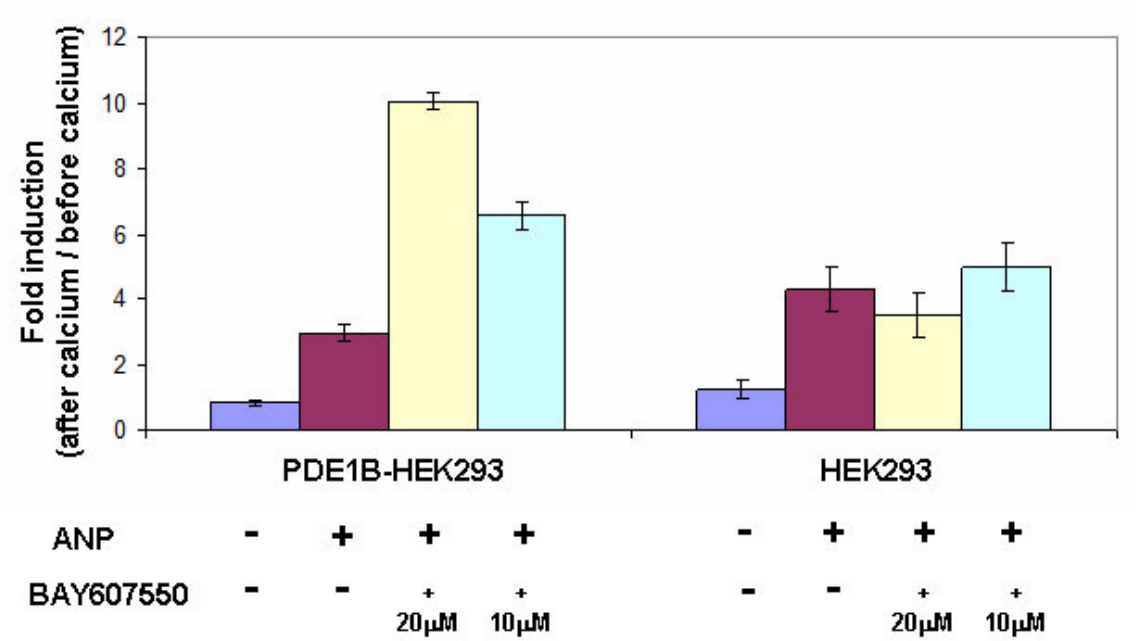
f. Dilute $CaCl_2$ (1M) in Ca^{2+} free assay buffer to 30 mM and add 10 µl / well. Measure the aequorin luminescence immediately. The luminescence intensity in the wells without cells is defined as background.

Figure 2. Inhibition of PDE1B activity by BAY607550 in PDE1B-HEK293 cells potentiated the ANP-induced cGMP level, resulting in calcium influx that stimulated luminescence signals.



PDE1B-HEK293 or HEK293 cells were transiently transfected with ANP receptor, CNGA2 and aequorin, and activated by ANP in the presence or in the absence of the PDE1B inhibitor BAY607550 as described in the protocol. Data were shown as fold induction of luminescence after addition of Ca^{2+} compared to values before addition of Ca^{2+}. Results showed that BAY607550 significantly potentiated the ANP-induced cGMP level in PDE1B-HEK293 cells but not in parental HEK293 cells, resulting in calcium influx that stimulated luminescence signals.

Figure 3. BAY607550 dose response in PDE1B-HEK293.

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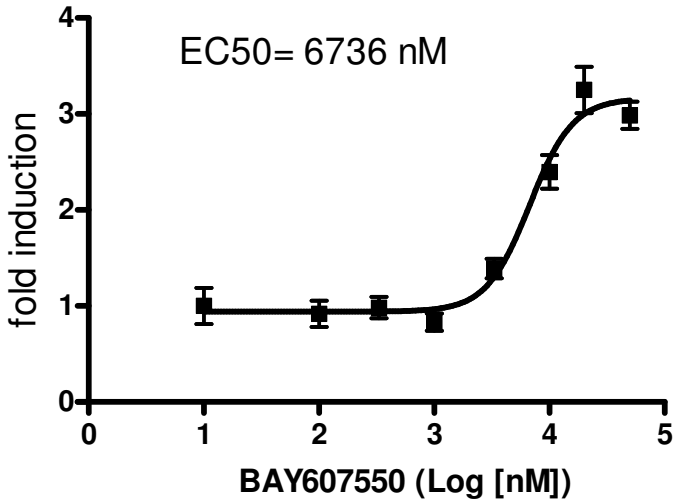

Results were shown as fold induction of luminescence intensity, determined by comparing values against the mean value for control cells without BAY607550 treatment. The inhibition of PDE1B in cells induces the luminescence, so the inhibitory effects of the compounds on PDE1B activity is expressed as EC50.

References

1. Yu, J. *et al.* (1997). "Identification and Characterization of a Human Calmodulin-Stimulated Phosphodiesterase PDE1B1 "*Cell Signaling* **9 (7):** 519-529.
2. Bender, A.T. *et al*., (2005). "Selective up-regulation of PDE1B2 upon monocyte-to-macrophage differentiation." *PNAS* **102 (2):** 497-502.
3. Wunder, F. *et al.,* (2009). "A novel PDE2A reporter cell line: characterization of the cellular activity of PDE inhibitors." *Mol. Pharm.* **6 (1):** 326-36.