PhotoScreen™ Double Transfected Cell Lines

CHO PhotoScreen™ P2RX1 Cell Line

PRODUCT NO.: AX-009-PCF
LOT NO.: 458-036-A

MATERIAL PROVIDED

CELLS: 2×1 mL frozen aliquots (AX-009-PCFV)
FORMAT: ~2.5×10^6 cells/mL in medium without antibiotics with 10% DMSO
STORAGE CONDITIONS: Store in liquid nitrogen

PRODUCT INFORMATION

CELLULAR BACKGROUND: CHO-K1

CELL LINE DEVELOPMENT: An expression vector containing the coding sequence of the human P2X_1 ion channel under the control of the CMV promoter was transfected in CHO-K1 cells stably expressing mitochondrially targeted Photina®. Resistant clones were obtained by limiting dilution and compared for their response to a reference agonist using the PhotoScreen™ assay.

DNA ACCESSION NUMBER: Identical to coding sequence of GenBank NM_002558.2

PROTEIN ACCESSION NUMBER: Identical to GenBank sequence NP_002549.1

MEMBRANE RECEPTOR (B_MAX): 31 pmol/mg

K_D FOR [^3H- ALPHA, BETA METHYLENE-ATP] : 13 nM

QUALITY CONTROL

REFERENCE AGONIST – ALPHA, BETA METHYLENE-ATP (EC_{50}): 16 nM (PHOTOSCREEN™ ASSAY)

STABILITY: Cells were kept in continuous culture for 20 passages and showed no significant decrease in functional response (EC_{50}, Emax).

MYCOPLASMA: The cell line tested negative for Mycoplasma.
Figure 1. PhotoScreen™ Agonist Dose-Response. Cells (10,000 cells/well) were seeded into the wells of a TC-treated black, clear bottom 384-well plate. Cells were loaded with 5 µM coelenterazine in HBSS for 4h at 37°C. Agonists, prepared in HBSS + 10 mM CaCl₂, were dispensed on the cells and signal was measured from seconds 0 to 50 following agonist addition using the LumiLux® Cellular Screening Platform. Data from a representative experiment are shown.

<table>
<thead>
<tr>
<th>Agonist</th>
<th>pEC₅₀</th>
<th>TOP Agonist (AUC, RLU)</th>
<th>% of Digitonin Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>alpha, beta methylene-ATP</td>
<td>7.9</td>
<td>1.31 x 10⁶</td>
<td>115%</td>
</tr>
<tr>
<td>beta, gamma methylene-ATP</td>
<td>7.0</td>
<td>1.17 x 10⁶</td>
<td>102%</td>
</tr>
<tr>
<td>2'(3')-O-(4-benzoylbenzoyl)ATP</td>
<td>7.6</td>
<td>1.12 x 10⁶</td>
<td>98%</td>
</tr>
</tbody>
</table>

Figure 2. PhotoScreen™ Antagonist Dose Response. Cells (10,000 cells/well) were seeded into the wells of a TC-treated black, clear bottom 384-well plate. Cells were loaded with 5 µM coelenterazine in HBSS for 4h at 37°C. Antagonists, prepared in HBSS, were added to the cells and, after 10 min, a concentration of 3 x EC₈₀ (final EC₈₀ concentration of 17.6 nM) of alpha, beta methylene-ATP, prepared in HBSS + 15 mM CaCl₂, was dispensed on the cells and signal was measured from seconds 0 to 50 following agonist addition using the LumiLux® Cellular Screening Platform. Data from a representative experiment are shown. It is to be noted that, although these 3 molecules have been reported to be antagonists for the P2X₁ receptor, some slow-acting agonist activity was observed for all 3 antagonists in the PhotoScreen assay.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>pIC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNT-ATP</td>
<td>8.2</td>
</tr>
<tr>
<td>PPNDS</td>
<td>7.5</td>
</tr>
<tr>
<td>NF279</td>
<td>6.5</td>
</tr>
</tbody>
</table>
Figure 3. Agonist dose-response measured by whole cell voltage clamp. Each current measurement required to establish the dose-response curve was preceded and followed by a measurement of the maximum current response to alpha, beta methylene-ATP or beta, gamma methylene-ATP as an internal control. Current measurements used for the dose-response curve were normalized to the respective flanking control current responses of 30 µM alpha, beta methylene-ATP or 100 µM beta, gamma methylene-ATP. No inward current was elicited when stimulating control parental cells with 100 µM alpha, beta methylene-ATP.

RECOMMENDED CELL CULTURE CONDITIONS

CELL CULTURE MEDIUM: Dulbecco’s MEM/ Nutrient Mix F12, 10% FBS; 1 mM sodium pyruvate, 100 IU/mL penicillin, 100 µg/mL streptomycin, and 500 µg/mL G418 (receptor expression selection); and 5 µg/mL puromycin (photoprotein expression selection)

THAWING CELLS: Using appropriate personal protective equipment, place the frozen ampoule in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray ampoule with 70% ethanol and wipe excess with a sterile towel. Under aseptic conditions using a pipette, transfer content to 10 mL complete medium and centrifuge (150 × g, 5 min). Resuspend cell pellet in 10 mL of complete medium and transfer to an appropriate culture flask (see table). Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO₂.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cells/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-K1</td>
<td>11 000 – 15 000</td>
</tr>
<tr>
<td>HEK-293</td>
<td>41 000 – 45 000</td>
</tr>
<tr>
<td>1321N1</td>
<td>19 000 – 23 000</td>
</tr>
</tbody>
</table>

CELL CULTURE PROTOCOL: Typically, for regular cell culture maintenance, cells are grown to 80% confluence, trypsinized (0.05% trypsin / 1 mM EDTA in calcium and magnesium-free PBS) and diluted 1/5. Under these conditions, cell passages should be carried out every 3-5 days.
**PhotoScreen™ ASSAY**

**EXPERIMENTAL PROCEDURE (ADHESION MODE):**

1. Cells grown till mid-log phase (70-90% confluency) are detached using trypsin and are seeded at a concentration of 10,000 cells/well in TC-treated black, clear bottom assay plates in culture medium without antibiotics and with 10% FBS. Cells are let to adhere in a 37°C, 5% CO₂ incubator overnight. **Note:** When working with the FLIPR TETRA®, white, clear bottom assay plates are used instead of black, clear bottom plates.

2. Medium is removed by plate overthow and tapping on a paper towel, then 20μL/well of HBSS +15 mM HEPES containing 10 μM (FLIPR TETRA®) or 5 μM (Lumilux Cellular Screening platform) native coelenterazine is added to the cells and plates are incubated for 4 h at 37°C in the dark.

3. For the agonist assay, using the reader’s automatic injection system, dispense per well in triplicate on the coelenterazine-loaded cells 20 μL of agonist at the desired concentrations, diluted in HBSS + 15 mM HEPES + 10 mM CaCl₂, and record the relative light emission for the desired time interval. Digitonin at a final concentration of 50 μM diluted in assay medium is used to measure the receptor independent cellular calcium response.

4. For the antagonist assay, dispense per well in triplicate on the coelenterazine-loaded cells 20 μL of antagonist at the desired concentrations, diluted in HBSS + 15 mM HEPES. After 10 min of incubation, using the reader’s automatic injection system, inject 20 μL of the reference agonist at a final concentration equivalent to the EC₈₀, prepared in HBSS + 15 mM HEPES + 15 mM CaCl₂, and record the relative light emission for the desired time interval.

**Note #1:** This assay has been validated also on the FLIPR TETRA® system in adherent mode.

**Note #2:** Using a suspension cells protocol with this cell line is not possible, as no response to P2X₁ agonists could be observed when working with cells in suspension.

**Note #3:** Agonist solutions are prepared with additional CaCl₂ (10 mM for agonist assays and 15 mM for antagonist assays) in order to increase the calcium flux through the P2X₁ channel and hence the signal intensity.

**REFERENCES**


**SUGGESTED MATERIAL AND INSTRUMENTATION**

### CELL CULTURE
- DMEM/F-12: Invitrogen 11320
- RPMI: Invitrogen 11875-135
- EMEM: Lonza BE06-174G
- Sodium Pyruvate: Invitrogen 11360
- PEN-STREP: Lonza DE17-602E
- Geneticin/G418: Invitrogen 11811-031
- Puromycin: Sigma-Aldrich P7255
- PBS: Lonza BE17-515Q

### PHOTOSCREEN™ ASSAY
- HBSS: Invitrogen 14025
- BSA, protease free: Sigma-Aldrich A3059
- Native Coelenterazine: Promega S2001
- Digitonin: Sigma-Aldrich 37006
- alpha, beta methylene-ATP: Sigma-Aldrich M-6517
- beta, gamma methylene-ATP: Sigma-Aldrich M7510
- 2'(3')-O-(4-Benzoylbenzoyl) ATP: Sigma-Aldrich B6396
- TNT-ATP: Tocris 2464
- PPNDS: Tocris 1309
- NF279: Tocris 1199

### BINDING ASSAY
- $^{3}$H- alpha, beta methylene-ATP: PerkinElmer NET1068

### MICROPLATES
- 384 well Optiplate™, white, opaque: PerkinElmer 6007290
- ViewPlate-384 F TC, black, clear bottom tissue culture treated, sterile, 384-well, with lid: PerkinElmer 6007460
- ViewPlate-1536 F TC, black, clear bottom, tissue culture treated, sterile, LoBase, 1536-well: PerkinElmer 6004460
- ViewPlate-384 TC, white, clear bottom, tissue culture treated, sterile, 384-well, with lid: PerkinElmer 6007480
- ViewPlate-1536 TC, white, clear bottom, tissue culture treated, with lid, sterile, LoBase 1536-well: PerkinElmer 6004480

### INSTRUMENTS (visit our web site or contact your local sales office for more information)
- MicroBeta® Jet: PerkinElmer 1450-024 + 1450-221
- EnVision® with Injectors: PerkinElmer 2103-0020 + 2203-1060
- Victor™ with Injectors: PerkinElmer 1420-060 / 1420-032 + 1420-2550
- LumiLux®-384 (110-120V, 60 Hz): PerkinElmer PTSSPL11
- LumiLux®-384 (220V, 50 Hz): PerkinElmer PTSSPL12
- LumiLux®-1536 (115V, 60 Hz): PerkinElmer PTSSPL21
- LumiLux®-1536 (230V, 50 Hz): PerkinElmer PTSSPL22

Trademark notices: The PerkinElmer logo and design, LumiLux, EnVision and MicroBeta are registered trademarks of PerkinElmer, Inc. Optiplate, PhotoScreen and Victor are trademarks of PerkinElmer, Inc. or its subsidiaries, in the United States and other countries. Photina is a registered trademark of Axxam S.p.A. FLIPR®TETRA is a registered trademark of MDS Analytical Technologies. All other trademarks not owned by PerkinElmer, Inc. or its subsidiaries that are depicted herein are the property of their respective owners.