human TRPA1 Ion Channel Cell Line

Product No.: AX-004-PCL

Lot No.: 2293079

Material Provided

Cells:	2 x 1 mL frozen aliquot (AX-004-PCLV)
Format:	~2.5 x 10^6 cells /mL in freezing medium

Product Information

Cellular Background:	HEK-293
Cell Line Development:	An expression vector containing the coding sequence of the human Transient Receptor Potential cation channel subfamily A member 1 (TRPA1) ion channel under the control of the CMV promoter was transfected in HEK-293 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 Sequence:	Identical to coding sequence of GenBank NM_007332.1
Corresponding Protein Sequence:	Identical to GenBank O75762.1
Receptor expression level (B_{max})	Not determined for this cell line.
Shipping Conditions:	Shipped on dry ice. Please ensure dry ice is still present in the package upon receipt or contact customer support.
Storage Conditions:	Store in liquid nitrogen (vapor phase) immediately upon receipt.

Quality Control

The EC_{50} value for a reference agonist was determined in a PhotoScreen® assay performed with adherent cells on a LumiLux® instrument. A mycoplasma test was performed using MycoAlert® Mycoplasma (Lonza) detection kit. We certify that these results meet our quality release criteria.

Trans-Cinnamaldehyde (EC_{50}):	N/A
Stability:	Cells were kept in continuous culture for 20 passages (~ 60 days) and showed no decrease in functional response in the PhotoScreen® assay (EC_{50}, E_{max}).
Mycoplasma:	This cell line tested negative for Mycoplasma.

Recommended Cell Culture Conditions

Complete Medium: MEM/EBSS, 10% fetal bovine serum (FBS), 400 ug/mL G418 sulfate.

Freezing Medium: MEM/EBSS, 10% fetal bovine serum (FBS) with 10% DMSO, without selection agents.

Thawing Cells: Using appropriate personal protective equipment, place the frozen aliquot in a 37°C water bath (do not submerge) and agitate until its content is thawed completely. Immediately remove from water bath, spray aliquot with 70% ethanol and wipe excess with a towel. Under aseptic conditions using a pipette, transfer content to 10 mL complete medium and centrifuge (150 *x g*, 5 min). Resuspend cell pellet in 10 mL of complete medium and transfer to an appropriate culture flask (see recommended seeding density below). Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO_2.

Recommended Seeding Density: 41,000 – 45,000 cells/cm^2

Cell Culture Protocol: Typically, for regular cell culture maintenance, these cells are grown to 80% confluence and trypsinized (0.05% trypsin / 0.5 mM EDTA in calcium and magnesium free HBSS). Under these conditions, cell passages should be carried out every 3-5 days.

Typical Product Data – PhotoScreen® Assay

Please refer to User Manual for culture conditions and detailed assay protocols.

Agonist	pEC_{50}	TOP Agonist (RLU)	% of Digitonin Response
***trans*-cinnamaldehyde**	3.6	166 000	80%
AITC	4.3	166 000	-
BITC	4.3	173 000	-

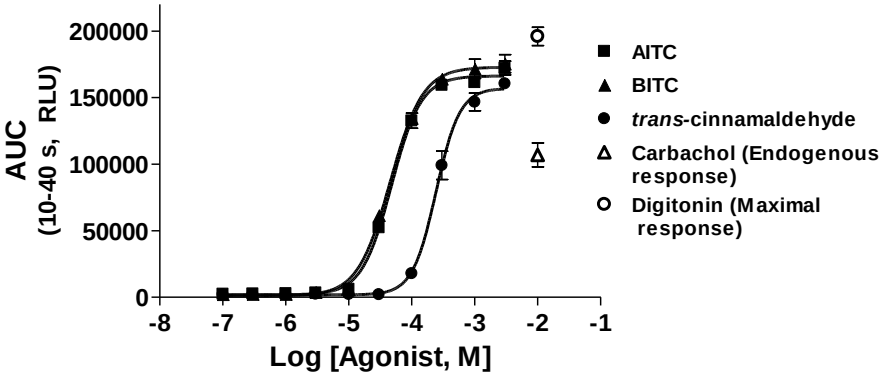

Figure 1. PhotoScreen® Agonist Dose Response. Coelenterazine-loaded cells (20,000 cells) were dispensed into the wells of a black, clear bottom 384-well plate containing the agonist dilutions. Signal was measured from 10 to 40 s following cell addition on the LumiLux® Cellular Screening Platform.

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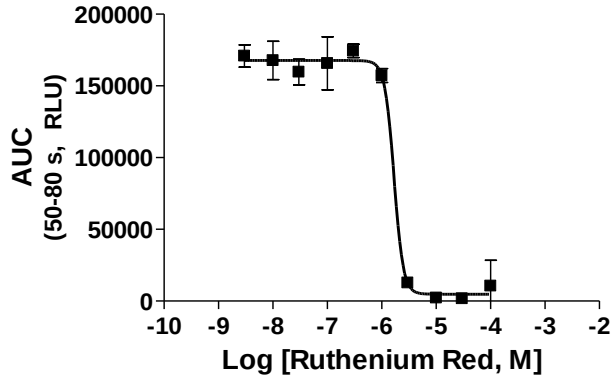

Antagonist	pIC_{50}
Ruthenium Red	5.8

Figure 2. PhotoScreen® Antagonist Dose Response. Coelenterazine-loaded cells (20,000 cells) were dispensed into the wells of a black, clear bottom 384-well plate containing the antagonist dilutions. After 10 min, the agonist (EC_{80} final concentration) was added and signal measured from 50 to 80 s following agonist addition on the LumiLux® Cellular Screening Platform.

References

1. Bovolenta S, Foti M, Lohmer S, Corazza S. (2007) Development of a Ca^{2+}-activated photoprotein, Photina®, and its application to high-throughput screening. J Biomol Screen. 12: 694-704.

2. Button D, Brownstein M. (1993) Aequorin-expressing mammalian cell lines used to report Ca^{2+} mobilization. Cell Calcium. 14: 663-671.

3. Sheu YA, Kricka LJ, Pritchett DB. (1993) Measurement of intracellular calcium using bioluminescent aequorin expressed in human cells. Anal Biochem. 209: 343-347.

4. Stables J, Green A, Marshall F, Fraser N, Knight E, Sautel M, Milligan G, Lee M, Rees S. (1997) A bioluminescent assay for agonist activity at potentially any G-protein-coupled receptor. Anal Biochem. 252: 115-126.

Materials and Instrumentation

	SUPPLIER	CATALOG #
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
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PHOTOSCREEN® ASSAY		
• DMEM/F-12	Invitrogen	11039
• BSA, protease free	Sigma-Aldrich	A3059
• Native Coelenterazine	Promega	S2001
• Digitonin	Sigma-Aldrich	37006
• Trans-cinnamaldehyde	Sigma-Aldrich	239 968

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