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PRODUCT DATASHEET

Ready-to-Assay™ PK2 Prokinecticin Receptor Frozen Cells

CATALOG NUMBER: HTS182RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

Prokineticins, also known as endocrine gland vascular endothelial growth factors (EG-VEGF), are two ~10 kD secreted proteins originally described to mediate angiogenesis and gastrointestinal smooth muscle contraction (Li et al., 2001; LeCouter et al., 2003). Subsequently, prokineticins have been found to mediate central nervous system functions including circadian rhythms and olfactory bulb development (Cheng et al., 2002; Ng et al., 2005). Two Gq-coupled receptors, PK1 and PK2 (also known as GPR73a and GPR73b), mediate cellular responses to prokineticins (Lin et al., 2002). The phenotype of the PK2-null mouse indicates that PK2 promotes development of the olfactory bulb and several male and female reproductive organs (Matsumoto et al., 2006). Cloned human PK2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PK2 expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between PK2 and its ligands.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

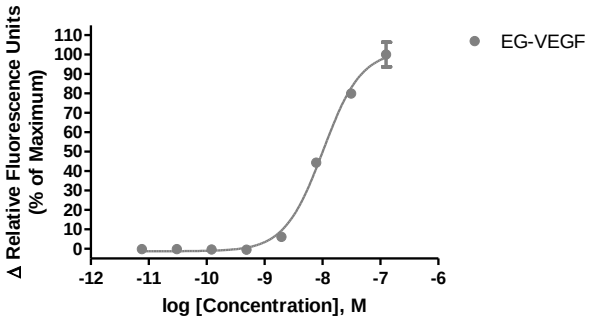

Figure 1. Representative data for activation of PK2 receptor. Calcium flux in PK2–expressing Chem-1 cell line induced by EG-VEGF PK2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,500 RLU (Relative Light Units).

Table 1. EC_{50} value of PK2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
EG-VEGF	Calcium Flux	10	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).

11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
EG-VEGF ligand	R&D Systems: 1209-EV
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

PK2 cDNA (Accession Number: NM_144773; see CODING SEQUENCE below) expressed from a proprietary E5 promoter plasmid.

CODING SEQUENCE

```
atggcagcccagaatggaaacaccagtttcacacccaactttaatccaccccaagaccat
 M  A  A  Q  N  G  N  T  S  F  T  P  N  F  N  P  P  Q  D  H
gcctcctccctctcctttaacttcagttatggtgattatgacctccctatggatgaggat
 A  S  S  L  S  F  N  F  S  Y  G  D  Y  D  L  P  M  D  E  D
gaggacatgaccaagacccggaccttcttcgcagccaagatcgtcattggcattgcactg
 E  D  M  T  K  T  R  T  F  F  A  A  K  I  V  I  G  I  A  L
gcaggcatcatgctggtctgcggcatcggtaactttgtctttatcgctgccctcacccgc
 A  G  I  M  L  V  C  G  I  G  N  F  V  F  I  A  A  L  T  R
tataagaagttgcgcaacctcaccaatctgctcattgccaacctggccatctccgacttc
 Y  K  K  L  R  N  L  T  N  L  L  I  A  N  L  A  I  S  D  F
ctggtggccatcatctgctgccccttcgagatggactactacgtggtacggcagctctcc
 L  V  A  I  I  C  C  P  F  E  M  D  Y  Y  V  V  R  Q  L  S
tgggagcatggccacgtgctctgtgcctccgtcaactacctgcgcaccgtctccctctac
 W  E  H  G  H  V  L  C  A  S  V  N  Y  L  R  T  V  S  L  Y
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 V  H  S  H  G  L  C  A  V  L  G  T  L  L  R  F  H  H  R  S
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 G  L  Y  Q  A  E  V
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS182M	ChemiScreen™ PK2 Prokinecticin receptor membrane prep

REFERENCES

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2. LeCouter J et al. (2003) Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. Nat. Med. 8: 913-7.
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4. Lin DC et al. (2002) Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. J. Biol. Chem. 277: 19276-80.
5. Matsumoto S et al. (2006) Abnormal development of the olfactory bulb and reproductive tissues in mice lacking prokineticin receptor PKR2. Proc. Natl. Acad. Sci. USA 103: 4140-4145.
6. Ng KL et al. (2005) Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. Science 308: 1923-7.

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