eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ GPR68/OGR1 Proton-Sensing Receptor Frozen Cells

CATALOG NUMBER: HTS153RTA

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.

GPR68, also known as OGR1 (ovarian cancer G-protein-coupled receptor 1), was initially thought to be a receptor for sphingosylphosphorylcholine, although these results are controversial. Subsequent studies indicated that GPR68/OGR1 functions as a G_q-coupled sensor for extracellular pH, with maximal signaling occurring at pH of 7 or below (Ludwig *et al*., 2003; Tomura *et al*., 2005). Expression of GPR68/OGR1 is upregulated during RANKL-induced osteoclast differentiation, and knockdown of GPR68/OGR1 expression inhibits this differentiation process (Yang *et al*., 2006). Cloned human GPR68-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant GPR68 expression on the cell surface and contains high levels of the promiscuous G protein $G\alpha 15$ to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between GPR68 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

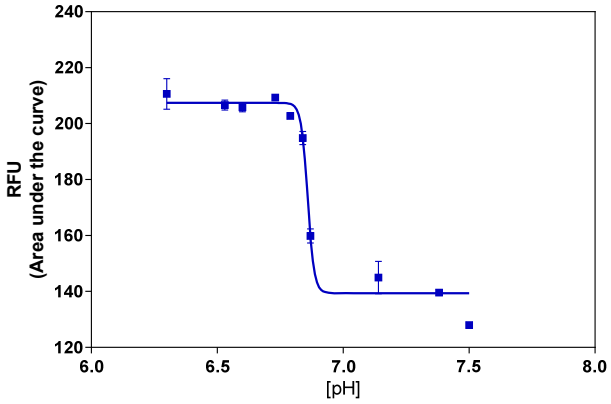

Figure 1. Representative data for activation of GPR68 receptor. Calcium flux in GPR68–expressing Chem-1 cell line induced by pH. GPR68–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}.

Table 1. Comparison of EC_{50} values of GPR68-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY	REFERENCE
pH	Calcium Flux	6.7 (pH)	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 wash 1X with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 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. Add Fluo-8 NW Ca^{2+} dye solution to assay plate (100µL/well for 96-well plate). Incubate for 1.5 hours in a humidified 37ºC 5% CO2 incubator.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well).
13. After 1.5 hour incubation remove Fluo-8 NW Ca^{2+} dye solution and replace with Asssay buffer (100µL/well).
14. 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
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
Dispense Height	25 µl
Dispense Speed	60 µl L/sec
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

GPR68 cDNA (Accession Number: NM_003485; see CODING SEQUENCE below) expressed from a proprietary E5 promoter plasmid.

CODING SEQUENCE

```
atgaggagtgtggccccttcaggcccaaagatggggaacatcactgcagacaactcctcg
M  R  S  V  A  P  S  G  P  K  M  G  N  I  T  A  D  N  S  S

atgagctgtaccatcgaccataccatccaccagacgctggccccggtggtctatgttacc
M  S  C  T  I  D  H  T  I  H  Q  T  L  A  P  V  V  Y  V  T

gtgctggtggtgggcttcccggccaactgcctgtccctctacttcggctacctgcagatc
V  L  V  V  G  F  P  A  N  C  L  S  L  Y  F  G  Y  L  Q  I

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atctgctcgctgcccttctggctgcagtacgtgctgcagcacgacaactggtctcacggc
I  C  S  L  P  F  W  L  Q  Y  V  L  Q  H  D  N  W  S  H  G

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D  L  S  C  Q  V  C  G  I  L  L  Y  E  N  I  Y  I  S  V  G

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F  L  C  C  I  S  V  D  R  Y  L  A  V  A  H  P  F  R  F  H

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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. Ludwig MG et al. (2003) Proton-sensing G-protein coupled receptors. Nature 425: 93-98.
2. Tomura H et al. (2005) Proton-sensing and lysolipid-sensitive G-protein-coupled receptors: A novel type of multi-functional receptors. Cell Signal. 17: 1466-1476.
3. Yang M et al. (2006) Expression of and role for ovarian cancer G-protein-coupled receptor 1 (OGR1) during osteoclastogenesis. J. Biol. Chem. 281: 23598-23605.

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