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PRODUCT DATASHEET

Ready-to-Assay™ OXGR1 α-Ketoglutarate Receptor Frozen Cells

CATALOG NUMBER: HTS191RTA

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.

OXGR1 (also known as GPR99 or GPR80) was originally identified as an orphan with a sequence most closely related to P2Y receptors. Studies to deorphanize OXGR1 revealed that α-ketoglutarate activates OXGR1 through Gq to increase intracellular calcium (He et al., 2004; Qi et al., 2004; Abbracchio et al., 2005). The EC50 of OXGR1 for α-ketoglutarate is in the μM range, similar to the concentration of α-ketoglutarate in the circulation, indicating that the interaction is physiologically significant. Cloned human OXGR1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant OXGR1-expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at OXGR1.

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

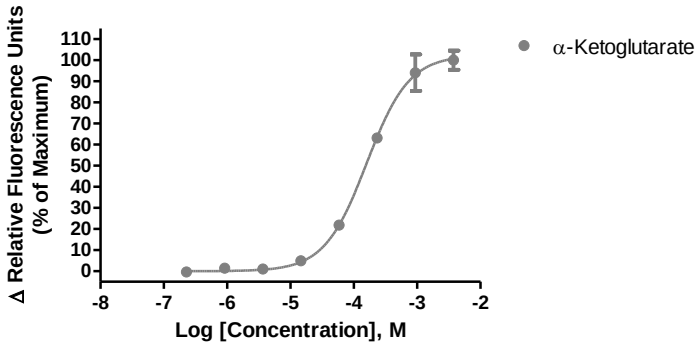

Figure 1. Representative data for activation of OXGR1 receptor. Calcium flux in OXGR1–expressing Chem-1 cell line induced by α-Ketoglutarate. OXGR1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand, 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,600 RLU (Relative Light Units).

Table 1. EC_{50} value of OXGR1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
α-Ketoglutarate	Calcium Flux	160	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
α-Ketoglutarate ligand	Sigma: K1875
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

Human OXGR1 cDNA (Accession Number: NM_080818.3; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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  1 - ATG AAT GAG CCA CTA GAC TAT TTA GCA AAT GCT TCT GAT TTC CCC GAT TAT GCA GCT GCT TTT GGA AAT  - 69
  1 -  M   N   E   P   L   D   Y   L   A   N   A   S   D   F   P   D   Y   A   A   A   F   G   N  - 23

 70 - TGC ACT GAT GAA AAC ATC CCA CTC AAG ATG CAC TAC CTC CCT GTT ATT TAT GGC ATT ATC TTC CTC GTG  - 138
 24 -  C   T   D   E   N   I   P   L   K   M   H   Y   L   P   V   I   Y   G   I   I   F   L   V  - 46

139 - GGA TTT CCA GGC AAT GCA GTA GTG ATA TCC ACT TAC ATT TTC AAA ATG AGA CCT TGG AAG AGC AGC ACC  - 207
 47 -  G   F   P   G   N   A   V   V   I   S   T   Y   I   F   K   M   R   P   W   K   S   S   T  - 69

208 - ATC ATT ATG CTG AAC CTG GCC TGC ACA GAT CTG CTG TAT CTG ACC AGC CTC CCC TTC CTG ATT CAC TAC  - 276
 70 -  I   I   M   L   N   L   A   C   T   D   L   L   Y   L   T   S   L   P   F   L   I   H   Y  - 92

277 - TAT GCC AGT GGC GAA AAC TGG ATC TTT GGA GAT TTC ATG TGT AAG TTT ATC CGC TTC AGC TTC CAT TTC  - 345
 93 -  Y   A   S   G   E   N   W   I   F   G   D   F   M   C   K   F   I   R   F   S   F   H   F  - 115

346 - AAC CTG TAT AGC AGC ATC CTC TTC CTC ACC TGT TTC AGC ATC TTC CGC TAC TGT GTG ATC ATT CAC CCA  - 414
116 -  N   L   Y   S   S   I   L   F   L   T   C   F   S   I   F   R   Y   C   V   I   I   H   P  - 138

415 - ATG AGC TGC TTT TCC ATT CAC AAA ACT CGA TGT GCA GTT GTA GCC TGT GCT GTG GTG TGG ATC ATT TCA  - 483
139 -  M   S   C   F   S   I   H   K   T   R   C   A   V   V   A   C   A   V   V   W   I   I   S  - 161

484 - CTG GTA GCT GTC ATT CCG ATG ACC TTC TTG ATC ACA TCA ACC AAC AGG ACC AAC AGA TCA GCC TGT CTC  - 552
162 -  L   V   A   V   I   P   M   T   F   L   I   T   S   T   N   R   T   N   R   S   A   C   L  - 184

553 - GAC CTC ACC AGT TCG GAT GAA CTC AAT ACT ATT AAG TGG TAC AAC CTG ATT TTG ACT GCA ACT ACT TTC  - 621
185 -  D   L   T   S   S   D   E   L   N   T   I   K   W   Y   N   L   I   L   T   A   T   T   F  - 207

622 - TGC CTC CCC TTG GTG ATA GTG ACA CTT TGC TAT ACC ACG ATT ATC CAC ACT CTG ACC CAT GGA CTG CAA  - 690
208 -  C   L   P   L   V   I   V   T   L   C   Y   T   T   I   I   H   T   L   T   H   G   L   Q  - 230

691 - ACT GAC AGC TGC CTT AAG CAG AAA GCA CGA AGG CTA ACC ATT CTG CTA CTC CTT GCA TTT TAC GTA TGT  - 759
231 -  T   D   S   C   L   K   Q   K   A   R   R   L   T   I   L   L   L   L   A   F   Y   V   C  - 253

760 - TTT TTA CCC TTC CAT ATC TTG AGG GTC ATT CGG ATC GAA TCT CGC CTG CTT TCA ATC AGT TGT TCC ATT  - 828
254 -  F   L   P   F   H   I   L   R   V   I   R   I   E   S   R   L   L   S   I   S   C   S   I  - 276

829 - GAG AAT CAG ATC CAT GAA GCT TAC ATC GTT TCT AGA CCA TTA GCT GCT CTG AAC ACC TTT GGT AAC CTG  - 897
277 -  E   N   Q   I   H   E   A   Y   I   V   S   R   P   L   A   A   L   N   T   F   G   N   L  - 299

898 - TTA CTA TAT GTG GTG GTC AGC GAC AAC TTT CAG CAG GCT GTC TGC TCA ACA GTG AGA TGC AAA GTA AGC  - 966
300 -  L   L   Y   V   V   V   S   D   N   F   Q   Q   A   V   C   S   T   V   R   C   K   V   S  - 322

967 - GGG AAC CTT GAG CAA GCA AAG AAA ATT AGT TAC TCA AAC AAC CCT TGA - 1014
323 -  G   N   L   E   Q   A   K   K   I   S   Y   S   N   N   P  Stp  - 345
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RELATED PRODUCTS

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

REFERENCES

1. Abbracchio MP *et al*. (2005) The recently deoprhanized GPR80 (GPR99) proposed to be the P2Y15 receptor is not a genuine P2Y receptor. *Trends Pharmacol. Sci.* 26: 8-9.
2. He W *et al.* (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* 429: 188-193.
3. Qi A-D *et al.* (2004) GPR80/99, proposed to be the P2Y15 receptor activated by adenosine and AMP, is not a P2Y receptor. *Purinergic Signalling* 1: 67-74.

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