eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ β_2 Adrenergic Receptor Frozen Cells

CATALOG NUMBER: HTS073RTA

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 over night recovery, assays for calcium response.

The endogenous catecholamines epinephrine, and norepinephrine have profound effects on smooth muscle activity, cardiac function, carbohydrate and fat metabolism, hormone secretion, neurotransmitter release, and central nervous system actions. These activities are mediated by GPCRs belonging to two subfamilies, the α- and β-adrenergic receptors (Bylund *et al*., 1994). The β-adrenergic receptors, primarily the β_2 subtype, mediate relaxation of smooth muscle in many tissues, and β_2-selective agonists are the preferred drugs for stimulating bronchodilation in the treatment of asthma and chronic obstructive pulmonary disease (Sears and Lotvall, 2005). Activation of the β-adrenergic receptors, primarily the β1 subtype and to a lesser extent the β2 subtype, acutely increases heart rate, cardiac output, and cardiac automaticity, and chronically increases cardiac myocyte apoptosis. As a result, β -adrenergic receptor antagonists (β blockers) are effective in the treatment of congestive heart failure and arrhythmia (Feldman *et al*., 2005). Cloned human β_2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant β_2 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 agonists, antagonists and modulators at β_2.

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.

APPLICATIONS

Calcium Flux Assays

Eurofins Pharma Bioanalytics Services US Inc.
15 Research Park Drive
St Charles MO 63304
USA
T | +1 844 522 7787
F | +1 636 362 7131
www.eurofins.com

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APPLICATION DATA

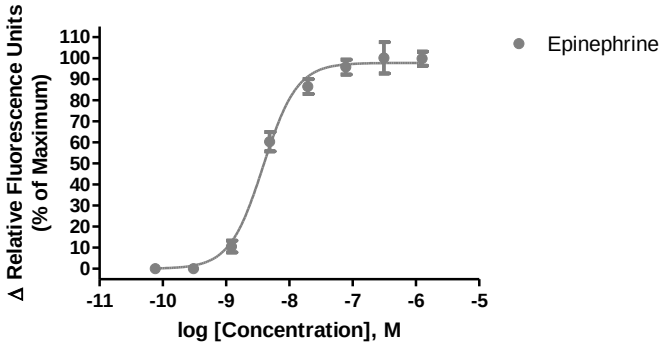

Figure 1. Representative data for activation of β_2 receptor. Calcium flux in β_2–expressing Chem-1 cell line induced by Epinephrine. β_2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 19,000 RLU (Relative Light Units).

Table 1. EC_{50} value of β_2-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Epinephrine	Calcium Flux	4	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: SH3026802
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Epinephrine ligand	Sigma: E1635
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

β_2 cDNA (Accession Number: NM_000024; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                ATG GGG CAA CCC GGG AAC GGC AGC GCC TTC TTG
                                                 M   G   Q   P   G   N   G   S   A   F   L

CTG GCA CCC AAT AGA AGC CAT GCG CCG GAC CAC GAC GTC ACG CAG CAA AGG GAC GAG GTG TGG GTG GTG
 L   A   P   N   G   S   H   A   P   D   H   D   V   T   Q   Q   R   D   E   V   W   V   V

GGC ATG GGC ATC GTC ATG TCT CTC ATC GTC CTG GCC ATC GTG TTT GGC AAT GTG CTG GTC ATC ACA GCC
 G   M   G   I   V   M   S   L   I   V   L   A   I   V   F   G   N   V   L   V   I   T   A

ATT GCC AAG TTC GAG CGT CTG CAG ACG GTC ACC AAC TAC TTC ATC ACT TCA CTG GCC TGT GCT GAT CTG
 I   A   K   F   E   R   L   Q   T   V   T   N   Y   F   I   T   S   L   A   C   A   D   L

GTC ATG GGC CTG GCA GTG GTG CCC TTT GGG GCC GCC CAT ATT CTT ATG AAA ATG TGG ACT TTT GGC AAC
 V   M   G   L   A   V   V   P   F   G   A   A   H   I   L   M   K   M   W   T   F   G   N

TTC TGG TGC GAG TTT TGG ACT TCC ATT GAT GTG CTG TGC GTC ACG GCC AGC ATT GAG ACC CTG TGC GTG
 F   W   C   E   F   W   T   S   I   D   V   L   C   V   T   A   S   I   E   T   L   C   V

ATC GCA GTG GAT CGC TAC TTT GCC ATT ACT TCA CCT TTC AAG TAC CAG AGC CTG CTG ACC AAG AAT AAG
 I   A   V   D   R   Y   F   A   I   T   S   P   F   K   Y   Q   S   L   L   T   K   N   K

GCC CGG GTG ATC ATT CTG ATG GTG TGG ATT GTG TCA GGC CTT ACC TCC TTC TTG CCC ATT CAG ATG CAC
 A   R   V   I   I   L   M   V   W   I   V   S   G   L   T   S   F   L   P   I   Q   M   H

TGG TAC CGG GCC ACC CAC CAG GAA GCC ATC AAC TGC TAT GCC AAT GAG ACC TGC TGT GAC TTC TTC ACG
 W   Y  R→R  A   T   H   Q   E   A   I   N   C   Y   A   N   E   T   C   C   D   F   F   T

AAC CAA GCC TAT GCC ATT GCC TCT TCC ATC GTG TCC TTC TAC GTT CCC CTG GTG ATC ATG GTC TTC GTC
 N   Q   A   Y   A   I   A   S   S   I   V   S   F   Y   V   P   L   V   I   M   V   F   V

TAC TCC AGG GTC TTT CAG GAG GCC AAA AGG CAG CTC CAG AAG ATT GAC AAA TCT GAG GGC CGC TTC CAT
 Y   S   R   V   F   Q   E   A   K   R   Q   L   Q   K   I   D   K   S   E   G   R   F   H

GTC CAG AAC CTT AGC CAG GTG GAG CAG GAT GGG CGG ACG GGG CAT GGA CTC CGC AGA TCT TCC AAG TTC
 V   Q   N   L   S   Q   V   E   Q   D   G   R   T   G   H   G   L   R   R   S   S   K   F

TGC TTG AAG GAG CAC AAA GCC CTC AAG ACG TTA GGC ATC ATC ATG GGC ACT TTC ACC CTC TGC TGG CTG
 C   L   K   E   H   K   A   L   K   T   L   G   I   I   M   G   T   F   T   L   C   W   L

CCC TTC TTC ATC GTT AAC ATT GTG CAT GTG ATC CAG GAT AAC CTC ATC CGT AAG GAA GTT TAC ATC CTC
 P   F   F   I   V   N   I   V   H   V   I   Q   D   N   L   I   R   K   E   V   Y   I   L

CTA AAT TGG ATA GGC TAT GTC AAT TCT GGT TTC AAT CCC CTT ATC TAC TGC CGG AGC CCA GAT TTC AGG
 L   N   W   I   G   Y   V   N   S   G   F   N   P   L   I   Y   C   R   S   P   D   F   R

ATT GCC TTC CAG GAG CTT CTG TGC CTG CGC AGG TCT TCT TTG AAG GCC TAT GGG AAT GGC TAC TCC AGC
 I   A   F   Q   E   L   L   C   L   R   R   S   S   L   K   A   Y   G   N   G   Y   S   S

AAC GGC AAC ACA GGG GAG CAG AGT GGA TAT CAC GTG GAA CAG GAG AAA GAA AAT AAA CTG CTG TGT GAA
 N   G   N   T   G   E   Q   S   G   Y   H   V   E   Q   E   K   E   N   K   L   L   C   E

GAC CTC CCA GGC ACG GAA GAC TTT GTG GGC CAT CAA GGT ACT GTG CCT AGC GAT AAC ATT GAT TCA CAA
 D   L   P   G   T   E   D   F   V   G   H   Q   G   T   V   P   S   D   N   I   D   S   Q

GGG AGG AAT TGT AGT ACA AAT GAC TCA CTG CTG TGA
 G   R   N   C   S   T   N   D   S   L   L  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS073M	ChemiScreen™ β_2 Adrenergic receptor membrane prep

REFERENCES

1. Bylund DB et al. (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46: 121-136.
2. Feldman DS et al. (2005) Mechanisms of Disease: β-adrenergic receptors—alterations in signal transduction and pharmacogenomics in heart failure. *Nat. Clin. Pract. Cardiovasc. Med*. 2: 475-83.
3. Sears MR and Lotvall J (2005) Past, present and future—β_2-adrenoceptor agonists in asthma management. *Respir. Med.* 99: 152-70.

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