eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ β_1 Adrenergic Receptor Frozen Cells

CATALOG NUMBER: HTS104RTA

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 three members of the β-adrenergic receptor family, β_1, β_2 and β_3, couple to G_s to increase cAMP upon activation. In the heart, the β_1 receptor constitutes 70-80% of the β-adrenergic receptors. Activation of cardiac β-adrenergic receptors, acutely increases heart rate, cardiac output, and cardiac automaticity, and chronically increases cardiac myocyte apoptosis. In failing hearts, the β_1 subtype is downregulated and desenstitized, probably as a result of increased catecholamine levels. As a result, β-adrenergic receptor antagonists (β blockers) are effective in the treatment of congestive heart failure and arrhythmia (Lohse et al., 2003). Millipore's cloned human β_1 expressing cell line is made in the Chem-1 host, which supports high levels of recombinant β_1 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_1.

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

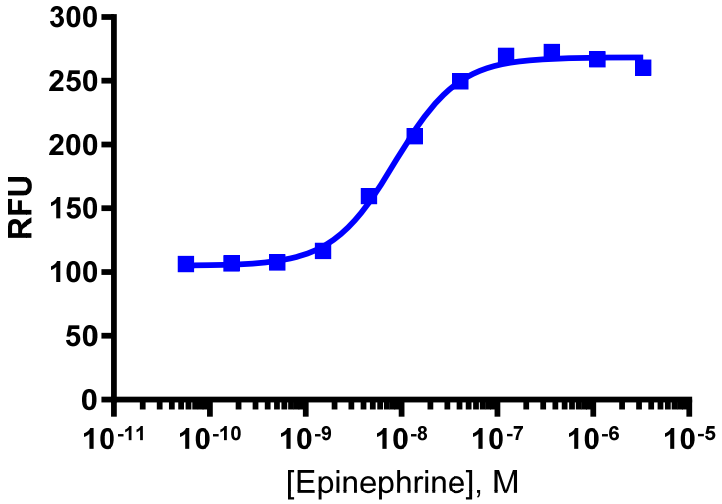

Figure 1. Representative data for activation of β_1 receptor. Calcium flux in β_1–expressing Chem-1 cell line induced by Epinephrine. B_1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 3-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}.

Table I. Comparison of EC_{50} values of β_1-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Epinephrine	Calcium Flux	8.6	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

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EXONGENOUS GENE EXPRESSION

ADRβ1 cDNA (Accession Number: NM_000684; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pBill plasmid.

CODING SEQUENCE

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ATG GGC GCG GGG GTG CTT GTC CTG GGC GCC TCC GAG CCC GGT AAC CTG TCG TCG GCC GCA CCG CTC CCC
 M   G   A   G   V   L   V   L   G   A   S   E   P   G   N   L   S   S   A   A   P   L   P

GAC GGC GCG GCC ACC GCG GCG CGG CTG CTG GTG CCC GCG TCG CCG CCC GCC TCG TTG CTG CCT CCC GCC
D   G   A   A   T   A   A   R   L   L   V   P   A   S   P   P   A   S   L   L   P   P   A

AGC GAA AGC CCC GAG CCG CTG TCT CAG CAG TGG ACA GCG GGC ATG GGT CTG CTG ATG GCG CTC ATC GTG
S   E   S   P   E   P   L   S   Q   Q   W   T   A   G   M   G   L   L   M   A   L   I   V

CTG CTC ATC GTG GCG GGC AAT GTG CTG GTG ATC GTG GCC ATC GCC AAG ACG CCG CGG CTG CAG ACG CTC
L   L   I   V   A   G   N   V   L   V   I   V   A   I   A   K   T   P   R   L   Q   T   L

ACC AAC CTC TTC ATC ATG TCC CTG GCC AGC GCC GAC CTG GTC ATG GGG CTG CTG GTG GTG CCG TTC GGG
T   N   L   F   I   M   S   L   A   S   A   D   L   V   M   G   L   L   V   V   P   F   G

GCC ACC ATC GTG GTG TGG GGC CGC TGG GAG TAC GGC TCC TTC TTC TGC GAG CTG TGG ACC TCA GTG GAC
A   T   I   V   V   W   G   R   W   E   Y   G   S   F   F   C   E   L   W   T   S   V   D

GTG CTG TGC GTG ACG GCC AGC ATC GAG ACC CTG TGT GTC ATT GCC CTG GAC CGC TAC CTC GCC ATC ACC
V   L   C   V   T   A   S   I   E   T   L   C   V   I   A   L   D   R   Y   L   A   I   T

TCG CCC TTC CGC TAC CAG AGC CTG CTG ACG CGC GCG CGG GCG CGG GGC CTC GTG TGC ACC GTG TGG GCC
S   P   F   R   Y   Q   S   L   L   T   R   A   R   A   R   G   L   V   C   T   V   W   A

ATC TCG GCC CTG GTG TCC TTC CTG CCC ATC CTC ATG CAC TGG TGG CGG GCG GAG AGC GAC GAG GCG CGC
I   S   A   L   V   S   F   L   P   I   L   M   H   W   W   R   A   E   S   D   E   A   R

CGC TGC TAC AAC GAC CCC AAG TGC TGC GAC TTC GTC ACC AAC CGG GCC TAC GCC ATC GCC TCG TCC GTA
R   C   Y   N   D   P   K   C   C   D   F   V   T   N   R   A   Y   A   I   A   S   S   V

GTC TCC TTC TAC GTG CCC CTG TGC ATC ATG GCC TTC GTG TAC CTG CGG GTG TTC CGC GAG GCC CAG AAG
V   S   F   Y   V   P   L   C   I   M   A   F   V   Y   L   R   V   F   R   E   A   Q   K


CAG GTG AAG AAG ATC GAC AGC TGC GAG CGC CGT TTC CTC GGC GGC CCA GCG CGG CCG CCC TCG CCC TCG
Q   V   K   K   I   D   S   C   E   R   R   F   L   G   G   P   A   R   P   P   S   P   S

CCC TCG CCC GTC CCC GCG CCC GCG CCG CCG CCC GGA CCC CCG CGC CCC GCC GCC GCC GCC GCC ACC GCC
P   S   P   V   P   A   P   A   P   P   P   G   P   P   R   P   A   A   A   A   A   T   A

CCG CTG GCC AAC GGG CGT GCG GGT AAG CGG CGG CCC TCG CGC CTC GTG GCC CTG CGC GAG CAG AAG GCG
P   L   A   N   G   R   A   G   K   R   R   P   S   R   L   V   A   L   R   E   Q   K   A

CTC AAG ACG CTG GGC ATC ATC ATG GGC GTC TTC ACG CTC TGC TGG CTG CCC TTC TTC CTG GCC AAC GTG
L   K   T   L   G   I   I   M   G   V   F   T   L   C   W   L   P   F   F   L   A   N   V

GTG AAG GCC TTC CAC CGC GAG CTG GTG CCC GAC CGC CTC TTC GTC TTC TTC AAC TGG CTG GGC TAC GCC
V   K   A   F   H   R   E   L   V   P   D   R   L   F   V   F   F   N   W   L   G   Y   A
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AAC TCG GCC TTC AAC CCC ATC ATC TAC TGC CGC AGC CCC GAC TTC CGC AAG GCC TTC CAG GGA CTG CTC
N   S   A   F   N   P   I   I   Y   C   R   S   P   D   F   R   K   A   F   Q   G   L   L

TGC TGC GCG CGC AGG GCT GCC CGC CGG CGC CAC GCG ACC CAC GGA GAC CGG CCG CGC GCC TCG GGC TGT
C   C   A   R   R   A   A   R   R   R   H   A   T   H   G   D   R   P   R   A   S   G   C

CTG GCC CGG CCC GGA CCC CCG CCA TCG CCC GGG GCC GCC TCG GAC GAC GAC GAC GAC GAT GTC GTC GGG
L   A   R   P   G   P   P   P   S   P   G   A   A   S   D   D   D   D   D   D   V   V   G

GCC ACG CCG CCC GCG CGC CTG CTG GAG CCC TGG GCC GGC TGC AAC GGC GGG GCG GCG GCG GAC AGC GAC
A   T   P   P   A   R   L   L   E   P   W   A   G   C   N   G   G   A   A   A   D   S   D

TCG AGC CTG GAC GAG CCG TGC CGC CCC GGC TTC GCC TCG GGA TCC AAG GTG TGA
S   S   L   D   E   P   C   R   P   G   F   A   S   G   S   K   V   Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS104M	ChemiScreen™ β_1 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. Lohse MJ et al. (2003) What is the role of β-adrenergic signaling in heart failure? Circ. Res. 93: 896-906.

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