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PRODUCT DATASHEET

Ready-to-Assay™ α_{1B} Adrenergic Family Receptor Frozen Cells

CATALOG NUMBER: HTS158RTA

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.

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-adrenoceptors (Bylund et al., 1994). The three members of the α_1 subclass of adrenoceptors, α_{1A}, α_{1B} and α_{1D}, couple to Gq, and promote contraction of vascular and urinary tract smooth muscle, relaxation of intestinal smooth muscle, increased contractile force in the heart, and glycogenolysis and gluconeogenesis in the liver. The different subtypes have overlapping distributions and variably contribute to these effects depending on species and tissue. Overexpression of a constitutively active α_{1B} mutant in the heart of transgenic mice resulted in cardiac hypertrophy with increased heart weight/body weight ratios. Analysis of α_{1B} knock out mice has provided evidence that α_{1B} is a mediator of blood pressure and aortic contractile responses induced by α_1 agonists (Milano et al., 1994). The locomotor and rewarding effects of pysochostimulants and opiates were suppressed in mice lacking α_{1B} -adrenergic receptors (Drouin et al. 2002). Cloned human α_{1B}-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant α_{1B} expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at α_{1B}.

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

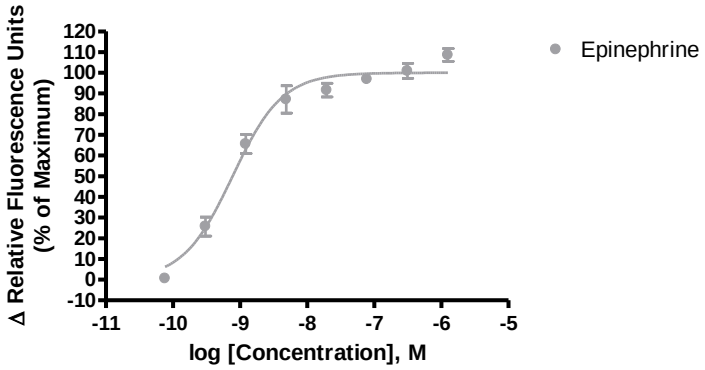

Figure 1. Representative data for activation of α_{1B} receptor. Calcium flux in α_{1B} –expressing Chem-1 cell line induced by epinephrine. α_{1B} –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 3,800 RLU (Relative Light Units).

Table 1. EC_{50} values of α_{1B} -expressing Chem-1 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Epinephrine	Calcium Flux	0.8	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
Epinephrine ligand	Sigma: 1635
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

ADRA1B cDNA (Accession Number: NM_000679.3; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG AAT CCC GAC CTG GAC ACC GGC CAC AAC ACA TCA GCA CCT GCC CAC TGG GGA GAG TTG AAA AAT GCC AAC TTC ACT
 M   N   P   D   L   D   T   G   H   N   T   S   A   P   A   H   W   G   E   L   K   N   A   N   F   T

GGC CCC AAC CAG ACC TCG AGC AAC TCC ACA CTG CCC CAG CTG GAC ATC ACC AGG GCC ATC TCT GTG GGC CTG GTG CTG
 G   P   N   Q   T   S   S   N   S   T   L   P   Q   L   D   I   T   R   A   I   S   V   G   L   V   L

GGC GCC TTC ATC CTC TTT GCC ATC GTG GGC AAC ATC CTA GTC ATC TTG TCT GTG GCC TGC AAC CGG CAC CTG CGG ACG
 G   A   F   I   L   F   A   I   V   G   N   I   L   V   I   L   S   V   A   C   N   R   H   L   R   T

CCC ACC AAC TAC TTC ATT GTC AAC CTG GCC ATG GCC GAC CTG CTG TTG AGC TTC ACC GTC CTG CCC TTC TCA GCG GCC
 P   T   N   Y   F   I   V   N   L   A   M   A   D   L   L   L   S   F   T   V   L   P   F   S   A   A

CTA GAG GTG CTC GGC TAC TGG GTG CTG GGG CGG ATC TTC TGT GAC ATC TGG GCA GCC GTG GAT GTC CTG TGC TGC ACA
 L   E   V   L   G   Y   W   V   L   G   R   I   F   C   D   I   W   A   A   V   D   V   L   C   C   T

GCG TCC ATT CTG AGC CTG TGC GCC ATC TCC ATC GAT CGC TAC ATC GGG GTG CGC TAC TCT CTG CAG TAT CCC ACG CTG
 A   S   I   L   S   L   C   A   I   S   I   D   R   Y   I   G   V   R   Y   S   L   Q   Y   P   T   L

GTC ACC CGG AGG AAG GCC ATC TTG GCG CTG CTC AGT GTC TGG GTC TTG TCC ACC GTC ATC TCC ATC GGG CCT CTC CTT
 V   T   R   R   K   A   I   L   A   L   L   S   V   W   V   L   S   T   V   I   S   I   G   P   L   L

GGG TGG AAG GAG CCG GCA CCC AAC GAT GAC AAG GAG TGC GGG GTC ACC GAA GAA CCC TTC TAT GCC CTC TTC TCC TCT
 G   W   K   E   P   A   P   N   D   D   K   E   C   G   V   T   E   E   P   F   Y   A   L   F   S   S

CTG GGC TCC TTC TAC ATC CCT CTG GCG GTC ATT CTA GTC ATG TAC TGC CGT GTC TAT ATA GTG GCC AAG AGA ACC ACC
 L   G   S   F   Y   I   P   L   A   V   I   L   V   M   Y   C   R   V   Y   I   V   A   K   R   T   T

AAG AAC CTA GAG GCA GGA GTC ATG AAG GAG ATG TCC AAC TCC AAG GAG CTG ACC CTG AGG ATC CAT TCC AAG AAC TTT
 K   N   L   E   A   G   V   M   K   E   M   S   N   S   K   E   L   T   L   R   I   H   S   K   N   F

CAC GAG GAC ACC CTT AGC AGT ACC AAG GCC AAG GGC CAC AAC CCC AGG AGT TCC ATA GCT GTC AAA CTT TTT AAG TTC
 H   E   D   T   L   S   S   T   K   A   K   G   H   N   P   R   S   S   I   A   V   K   L   F   K   F

TCC AGG GAA AAG AAA GCA GCT AAG ACG TTG GGC ATT GTG GTC GGT ATG TTC ATC TTG TGC TGG CTA CCC TTC TTC ATC
 S   R   E   K   K   A   A   K   T   L   G   I   V   V   G   M   F   I   L   C   W   L   P   F   F   I

GCT CTA CCG CTT GGC TCC TTG TTC TCC ACC CTG AAG CCC CCC GAC GCC GTG TTC AAG GTG GTG TTC TGG CTG GGC TAC
 A   L   P   L   G   S   L   F   S   T   L   K   P   P   D   A   V   F   K   V   V   F   W   L   G   Y

TTC AAC AGC TGC CTC AAC CCC ATC ATC TAC CCA TGC TCC AGC AAG GAG TTC AAG CGC GCT TTC GTG CGC ATC CTC GGG
 F   N   S   C   L   N   P   I   I   Y   P   C   S   S   K   E   F   K   R   A   F   V   R   I   L   G

TGC CAG TGC CGC GGC CGC GGC CGC CGC CGA CGC CGC CGC CGC CGT CGC CTG GGC GGC TGC GCC TAC ACC TAC CGG CCG
 C   Q   C   R   G   R   G   R   R   R   R   R   R   R   R   L   G   G   C   A   Y   T   Y   R   P

TGG ACG CGC GGC GGC TCG CTG GAG CGC TCG CAG TCG CGC AAG GAC TCG CTG GAC GAC AGC GGC AGC TGC CTG AGC GGC
 W   T   R   G   G   S   L   E   R   S   Q   S   R   K   D   S   L   D   D   S   G   S   C   L   S   G

AGC CAG CGG ACC CTG CCC TCG GCC TCG CCG AGC CCG GGC TAC CTG GGC CGC GGC GCG CCA CCG CCA GTC GAG CTG TGC
 S   Q   R   T   L   P   S   A   S   P   S   P   G   Y   L   G   R   G   A   P   P   P   V   E   L   C

GCC TTC CCC GAG TGG AAG GCG CCC GGC GCC CTC CTG AGC CTG CCC GCG CCT GAG CCC CCC GGC CGC CGC GGC CGC CAC
 A   F   P   E   W   K   A   P   G   A   L   L   S   L   P   A   P   E   P   P   G   R   R   G   R   H

GAC TCG GGC CCG CTC TTC ACC TTC AAG CTC CTG ACC GAG CCC GAG AGC CCC GGG ACC GAC GGC GGC GCC AGC AAC GGA
 D   S   G   P   L   F   T   F   K   L   L   T   E   P   E   S   P   G   T   D   G   G   A   S   N   G

GGC TGC GAG GCC GCG GCC GAC GTG GCC AAC GGG CAG CCG GGC TTC AAA AGC AAC ATG CCC CTG GCG CCC GGG CAG TTT
 G   C   E   A   A   A   D   V   A   N   G   Q   P   G   F   K   S   N   M   P   L   A   P   G   Q   F

TGA
Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS158M	ChemiScreen™ α_{1B} Adrenergic Family Receptor membrane prep

REFERENCES

1. Bylund DB *et al.* (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev.* 46: 121-136.
2. Cavalli A *et al.* (1997) Decreased blood pressure response in mice deficient of the a_{1B}-AR. *Proc. Natl. Acad. Sci. USA* 94: 11589–11594.
3. Milano CA *et al.* (1994) Myocardial expression of a constitutively active α_{1B}-adrenergic receptor in transgenic mice induces cardiac hypertrophy. *Proc. Natl. Acad. Sci. USA* 91: 10109-10113.

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