eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ $\alpha_{2\beta}$ Adrenergic Receptor Frozen Cells

CATALOG NUMBER: HTS157LRTA

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. ChemiBrite cells co-express a GPCR along with a novel variant of clytin, a calcium-activated photoprotein, to enable sensitive luminescent detection of ligand-induced calcium flux. The ChemiBrite version of clytin contains a mutation that increases its affinity for calcium to a level that permits detection of cytosolic calcium in many cells with greater sensitivity than other mitochondrially expressed photoproteins. Luminescent calcium assays offer several advantages over fluorescent calcium assays including; lower substrate cost, increased sensitivity, and lack of interference from fluorescent compounds.

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 α_2 adrenergic receptor subfamily members, consisting of α_{2A}, α_{2B}, and α_{2C}, couple primarily to G_i to inhibit cAMP production and play an important role in regulation of cardiovascular and CNS function. Experiments with $\alpha_{2\beta}$-selective agonists and mice lacking α_{2B} demonstrate that α_{2B} plays a role in salt-induced hypertension. Also, the difficulty in breeding homozygous α_{2B}-KO mice indicates the gene may additionally play an as-yet-unknown role in development or reproduction (Kable *et al.*,2000). Cloned $\alpha_{2\beta}$ receptor-expressing ChemiBrite cells were made by stable transfection of HEK293 cells with ChemiBrite clytin and the receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. The cells have been cryopreserved at an optimal time post-transfection. Upon thaw, recovery, and loading, the cells are ready for luminescent, fluorescent and cAMP accumulation analysis of agonists, antagonists and modulators at the $\alpha_{2\beta}$ receptor.

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: Luminescent Mode and Fluorescent Mode

APPLICATION DATA

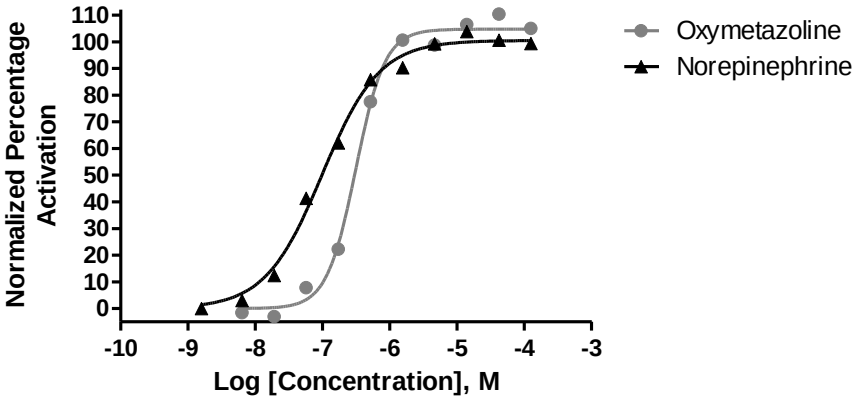

Figure 1. Representative data for activation of $\alpha_{2\beta}$ receptor. Calcium flux in $\alpha_{2\beta}$–expressing HEK293 cell line induced by Oxymetazoline. $\alpha_{2\beta}$–expressing HEK293 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 8,000 RLU (Relative Light Units).

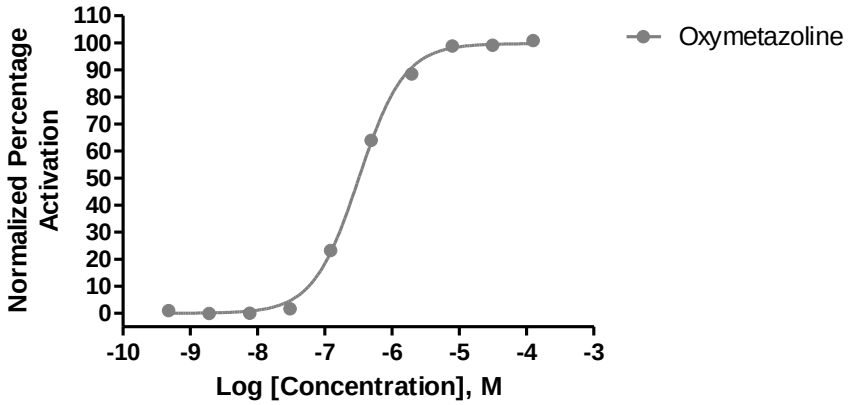

Figure 2. Representative data for activation of $\alpha_{2\beta}$ receptor expressed in HEK293 cells induced by Oxymetazoline using a luminescent calcium flux assay. $\alpha_{2\beta}$–expressing HEK293 cells were loaded with 10µM coelenterazine for 2h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format. Luminescence signal obtained in this experiment was 40,000 RLU (Relative Light Units) as measured by area-under-curve for 80s post agonist addition using the provided protocol.

Table 1. Comparison of EC_{50} values of $\alpha_{2\beta}$-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Oxymetazoline	Calcium Flux - Fluorescence	310	Eurofins Internal Data
Norepinephrine	Calcium Flux - Fluorescence	100	Eurofins Internal Data
Oxymetazoline	Calcium Flux - Luminescence	320	Eurofins Internal Data

ASSAY SETUP

Luminescence

Table 2. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Luminescence
Ex/Em	None/None
Camera Gain	280,000
Gate Open	100 %
Exposure Time	0.9 sec
Read Interval	1 sec.
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Fluorescence

Table 3. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 4. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Oxymetazoline ligand	Tocris: 1142
Norepinephrine ligand	Sigma: A7257
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/3574
Black (clear Bottom) cell assay plates	Corning: 3904/3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Luminescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC, $^{5e6/}_{5e5/ml}$ =10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, pH 7.4) and Loading buffer (Assay buffer with 10µM coelenterazine). *Note: Please prepare coelenterazine stock according to Manufacturer's Recommendations at 10mM to allow for 1:1000 dilution into Loading buffer (10uM final concentration). It is critical that coelenterazine solution is prepared at room temperature and is protected from light.*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. Export data to analyze using the area under the curve statistic.

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC, $^{5e6/}_{5e5/ml}$ =10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

HEK293

EXONGENOUS GENE EXPRESSION

Human ADRA2B cDNA (Accession Number: NM_000682; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein and promiscuous G protein expressed in a bicistronic vector

CODING SEQUENCE

```
ATG GAC CAC CAG GAC CCC TAC TCC GTG CAG GCC ACA GCG GCC ATA GCG GCG GCC ATC ACC TTC CTC
 M   D   H   Q   D   P   Y   S   V   Q   A   T   A   A   I   A   A   A   I   T   F   L

ATT CTC TTT ACC ATC TTC GGC AAC GCT CTG GTC ATC CTG GCT GTG TTG ACC AGC CGC TCG CTG CGC
 I   L   F   T   I   F   G   N   A   L   V   I   L   A   V   L   T   S   R   S   L   R

GCC CCT CAG AAC CTG TTC CTG GTG TCG CTG GCC GCC GCC GAC ATC CTG GTG GCC ACG CTC ATC ATC
 A   P   Q   N   L   F   L   V   S   L   A   A   A   D   I   L   V   A   T   L   I   I

CCT TTC TCG CTG GCC AAC GAG CTG CTG GGC TAC TGG TAC TTC CGG CGC ACG TGG TGC GAG GTG TAC
 P   F   S   L   A   N   E   L   L   G   Y   W   Y   F   R   R   T   W   C   E   V   Y

CTG GCG CTC GAC GTG CTC TTC TGC ACC TCG TCC ATC GTG CAC CTG TGC GCC ATC AGC CTG GAC CGC
 L   A   L   D   V   L   F   C   T   S   S   I   V   H   L   C   A   I   S   L   D   R

TAC TGG GCC GTG AGC CGC GCG CTG GAG TAC AAC TCC AAG CGC ACC CCG CGC CGC ATC AAG TGC ATC
 Y   W   A   V   S   R   A   L   E   Y   N   S   K   R   T   P   R   R   I   K   C   I

ATC CTC ACT GTG TGG CTC ATC GCC GCC GTC ATC TCG CTG CCG CCC CTC ATC TAC AAG GGC GAC CAG
 I   L   T   V   W   L   I   A   A   V   I   S   L   P   P   L   I   Y   K   G   D   Q

GGC CCC CAG CCG CGC GGG CGC CCC CAG TGC AAG CTC AAC CAG GAG GCC TGG TAC ATC CTG GCC TCC
 G   P   Q   P   R   G   R   P   Q   C   K   L   N   Q   E   A   W   Y   I   L   A   S

AGC ATC GGA TCT TTC TTT GCT CCT TGC CTC ATC ATG ATC CTT GTC TAC CTG CGC ATC TAC CTG ATC
 S   I   G   S   F   F   A   P   C   L   I   M   I   L   V   Y   L   R   I   Y   L   I

GCC AAA CGC AGC AAC CGC AGA GGT CCC AGG GCC AAG GGG GGG CCT GGG CAG GGT GAG TCC AAG CAG
 A   K   R   S   N   R   R   G   P   R   A   K   G   G   P   G   Q   G   E   S   K   Q

CCC CGA CCC GAC CAT GGT GGG GCT TTG GCC TCA GCC AAA CTG CCA GCC CTG GCC TCT GTG GCT TCT
 P   R   P   D   H   G   G   A   L   A   S   A   K   L   P   A   L   A   S   V   A   S

GCC AGA GAG GCC AAC GGA CAC TCG AAG TCC ACT GGG GAG AAG GAG GAG GGG GAG ACC CCT GAA GAT
 A   R   E   A   N   G   H   S   K   S   T   G   E   K   E   E   G   E   T   P   E   D

ACT GGG ACC CGG GCC TTG CCA CCC AGT TGG GCT GCC CTT CCC AAC TCA GGC CAG GGC CAG AAG GAG
 T   G   T   R   A   L   P   P   S   W   A   A   L   P   N   S   G   Q   G   Q   K   E

GGT GTT TGT GGG GCA TCT CCA GAG GAT GAA GCT GAA GAG GAG GAA GAG GAG GAG GAG GAG GAG GAA
 G   V   C   G   A   S   P   E   D   E   A   E   E   E   E   E   E   E   E   E   E   E

GAG TGT GAA CCC CAG GCA GTG CCA GTG TCT CCG GCC TCA GCT TGC AGC CCC CCG CTG CAG CAG CCA
 E   C   E   P   Q   A   V   P   V   S   P   A   S   A   C   S   P   P   L   Q   Q   P

CAG GGC TCC CGG GTG CTG GCC ACC CTA CGT GGC CAG GTG CTC CTG GGC AGG GGC GTG GGT GCT ATA
 Q   G   S   R   V   L   A   T   L   R   G   Q   V   L   L   G   R   G   V   G   A   I

GGT GGG CAG TGG TGG CGT CGA CGG GCG CAG CTG ACC CGG GAG AAG CGC TTC ACC TTC GTG CTG GCT
 G   G   Q   W   W   R   R   R   A   Q   L   T   R   E   K   R   F   T   F   V   L   A

GTG GTC ATT GGC GTT TTT GTG CTC TGC TGG TTC CCC TTC TTC TTC AGC TAC AGC CTG GGC GCC ATC
 V   V   I   G   V   F   V   L   C   W   F   P   F   F   F   S   Y   S   L   G   A   I

TGC CCG AAG CAC TGC AAG GTG CCC CAT GGC CTC TTC CAG TTC TTC TTC TGG ATC GGC TAC TGC AAC
 C   P   K   H   C   K   V   P   H   G   L   F   Q   F   F   F   W   I   G   Y   C   N

AGC TCA CTG AAC CCT GTT ATC TAC ACC ATC TTC AAC CAG GAC TTC CGC CGT GCC TTC CGG AGG ATC
 S   S   L   N   P   V   I   Y   T   I   F   N   Q   D   F   R   R   A   F   R   R   I

CTG TGC CGC CCG TGG ACC CAG ACG GCC TGG TGA
 L   C   R   P   W   T   Q   T   A   W  STP
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSHEK-3L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gαqi
HTS157L	ChemiBrite™ $\alpha_{2\beta}$ Adrenergic receptor stable cell line
HTS158C	ChemiScreen™ α_{1B} Adrenergic receptor stable cell line
HTS216C	ChemiScreen™ α_{1D} Adrenergic receptor stable cell line
HTS096C	ChemiScreen™ α_{2A} Adrenergic receptor stable cell line
HTS087C	ChemiScreen™ α_{1A} Adrenergic receptor stable cell line
HTS104C	ChemiScreen™ β_1 Adrenergic receptor stable cell line
HTS073C	ChemiScreen™ β_2 Adrenergic receptor stable cell line
HTS159C	ChemiScreen™ β_3 Adrenergic receptor stable cell line

REFERENCES

1. Bylund DB *et al*. (1994). IV. International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacol. Rev*. 46: 121-136.
2. Kable JW *et al*. (2000) In vivo gene modification elucidates subtype-specific functions of β2-adrenergic receptors. *J. Pharmacol. Exp. Ther*. 293: 1-7

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