eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ $CysLT_1$ Leukotriene Receptor Frozen Cells

CATALOG NUMBER: HTS061LRTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial.

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.

ChemiBrite™ cells express 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 photoproteins targeted to the mitochondria. Luminescent calcium assays offer several advantages over fluorescent calcium assays including increased sensitivity and lack of interference from fluorescent compounds.

The cysteinyl leukotrienes, leukotriene C4, leukotriene D4 and leukotriene E4, are arachidonic acid derivatives modified by glutathione, Cys-Gly or Cys. Activated mast cells release cysteinyl leukotrienes, which cause smooth muscle contraction, airway constriction, and vascular permeability. The biological effects of the cysteinyl leukotrienes are mediated by two GPCRs, CysLT1 and CysLT2. A CysLT1 selective antagonist, montelukast, is used clinically in the treatment of asthma (Brink et al., 2003; Evans, 2002). Cloned human CysLT1 receptor-expressing ChemiBrite™ cells were made by stable transfection of HEK293 cells with ChemiBrite™ clytin and the CysLT1 receptor. These stability-tested cells are ready for luminescent analysis of agonists, antagonists and modulators at the CysLT1 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

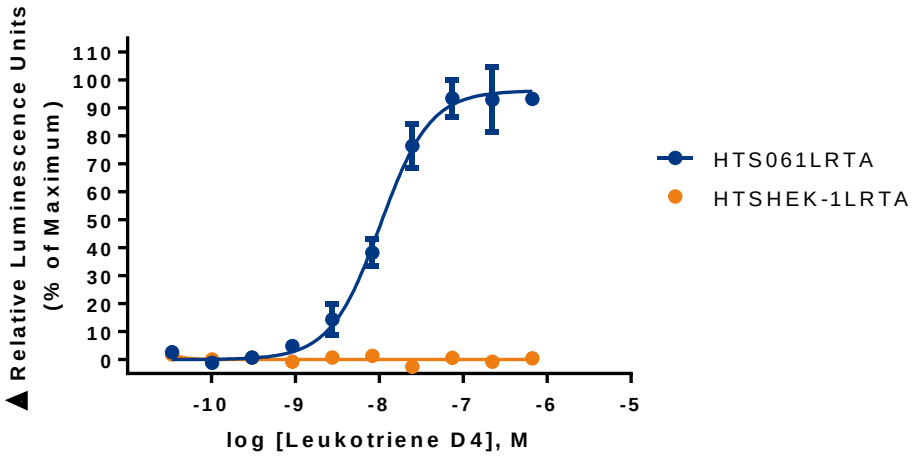

Figure 1. Representative data for activation of $CysLT_1$ receptor stably expressed in HEK293 cells induced by LTD_4 using a luminescent calcium flux assay. $CysLT_1$–expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 30,000 RLU (Relative Light Units) as measured by AUC (area under curve) for 80 s post agonist addition using the provided protocol. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

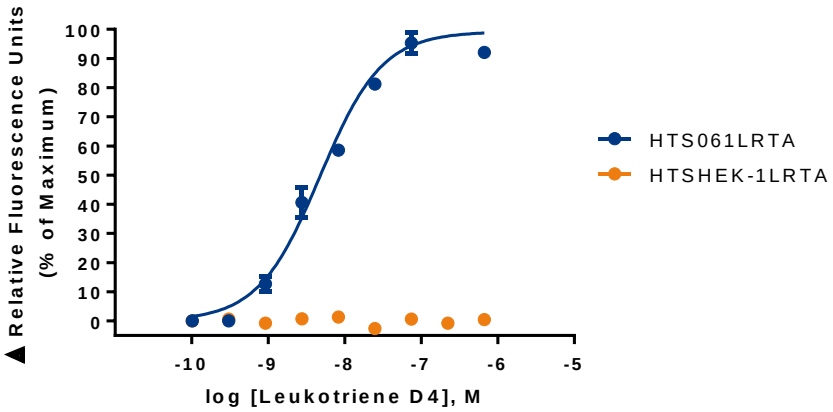

Figure 2. Representative data for activation of $CysLT_1$ receptor stably expressed in HEK293 cells induced by LTD_4 using a fluorescent calcium flux assay. $CysLT_1$-expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a calcium dye. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 3,000 RLU. Similarly parental cells (catalog #: HTSHEK-1L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of $CysLT_1$-expressing HEK293 cells

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
LTD_4	Calcium Flux - Luminescence	20	Eurofins Internal Data
LTD_4	Calcium Flux - Fluorescence	3.0	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

Table 3. Luminescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Quest Fluo-8™, AM	AAT Bioquest: 21080
Leukotriene D4 ligand	Cayman: 20310
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

Fluorescence

Table 4. 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 5. Fluorescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Leukotriene D4 ligand	Cayman: 20310
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

Assay Protocol – Luminescence

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% CO_2 incubator for 18-24 h.
9. 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.*
10. Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media Component.
11. 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.
12. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
13. 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.
14. 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. 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. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
8. 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*
9. Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media

Component.

10. 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.
11. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
12. 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.
13. 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 CYSLTR1 cDNA (Accession Number: NM_006639; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein expressed in a bicistronic vector

CODING SEQUENCE

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ATG GAT GAA ACA GGA AAT CTG ACA GTA TCT TCT GCC ACA TGC CAT GAC ACT ATT GAT GAC TTC CGC
 M   D   E   T   G   N   L   T   V   S   S   A   T   C   H   D   T   I   D   D   F   R

AAT CAA GTG TAT TCC ACC TTG TAC TCT ATG ATC TCT GTT GTA GGC TTC TTT GGC AAT GGC TTT GTG
 N   Q   V   Y   S   T   L   Y   S   M   I   S   V   V   G   F   F   G   N   G   F   V

CTC TAT GTC CTC ATA AAA ACC TAT CAC AAG AAG TCA GCC TTC CAA GTA TAC ATG ATT AAT TTA GCA
 L   Y   V   L   I   K   T   Y   H   K   K   S   A   F   Q   V   Y   M   I   N   L   A

GTA GCA GAT CTA CTT TGT GTG TGC ACA CTG CCT CTC CGT GTG GTC TAT TAT GTT CAC AAA GGC ATT
 V   A   D   L   L   C   V   C   T   L   P   L   R   V   V   Y   Y   V   H   K   G   I

TGG CTC TTT GGT GAC TTC TTG TGC CGC CTC AGC ACC TAT GCT TTG TAT GTC AAC CTC TAT TGT AGC
 W   L   F   G   D   F   L   C   R   L   S   T   Y   A   L   Y   V   N   L   Y   C   S

ATC TTC TTT ATG ACA GCC ATG AGC TTT TTC CGG TGC ATT GCA ATT GTT TTT CCA GTC CAG AAC ATT
 I   F   F   M   T   A   M   S   F   F   R   C   I   A   I   V   F   P   V   Q   N   I

AAT TTG GTT ACA CAG AAA AAA GCC AGG TTT GTG TGT GTA GGT ATT TGG ATT TTT GTG ATT TTG ACC
 N   L   V   T   Q   K   K   A   R   F   V   C   V   G   I   W   I   F   V   I   L   T

AGT TCT CCA TTT CTA ATG GCC AAA CCA CAA AAA GAT GAG AAA AAT AAT ACC AAG TGC TTT GAG CCC
 S   S   P   F   L   M   A   K   P   Q   K   D   E   K   N   N   T   K   C   F   E   P

CCA CAA GAC AAT CAA ACT AAA AAT CAT GTT TTG GTC TTG CAT TAT GTG TCA TTG TTT GTT GGC TTT
 P   Q   D   N   Q   T   K   N   H   V   L   V   L   H   Y   V   S   L   F   V   G   F

ATC ATC CCT TTT GTT ATT ATA ATT GTC TGT TAC ACA ATG ATC ATT TTG ACC TTA CTA AAA AAA TCA
 I   I   P   F   V   I   I   I   V   C   Y   T   M   I   I   L   T   L   L   K   K   S

ATG AAA AAA AAT CTG TCA AGT CAT AAA AAG GCT ATA GGA ATG ATC ATG GTC GTG ACC GCT GCC TTT
 M   K   K   N   L   S   S   H   K   K   A   I   G   M   I   M   V   V   T   A   A   F

TTA GTC AGT TTC ATG CCA TAT CAT ATT CAA CGT ACC ATT CAC CTT CAT TTT TTA CAC AAT GAA ACT
 L   V   S   F   M   P   Y   H   I   Q   R   T   I   H   L   H   F   L   H   N   E   T

AAA CCC TGT GAT TCT GTC CTT ACA ATG CAG AAG TCC GTG GTC ATA ACC TTG TCT CTG GCT GCA TCC
 K   P   C   D   S   V   L   T   M   Q   K   S   V   V   I   T   L   S   L   A   A   S

AAT TGT TGC TTT GAC CCT CTC CTA TAT TTC TTT TCT GGG GGT AAC TTT AGG AAA AGG CTG TCT ACA
 N   C   C   F   D   P   L   L   Y   F   F   S   G   G   N   F   R   K   R   L   S   T

TTT AGA AAG CAT TCT TTG TCC AGC GTG ACT TAT GTA CCC AGA AAG AAG GCC TCT TTG CCA GAA AAA
 F   R   K   H   S   L   S   S   V   T   Y   V   P   R   K   K   A   S   L   P   E   K

GGA GAA GAA ATA TGT AAA GTA TGA
 G   E   E   I   C   K   V   .
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSHEK-1L	ChemiBrite™ HEK293 Parental Stable Cell Line
HTS061L	ChemiBrite™ $CysLT_1$ Leukotriene Stable Cell Line
HTS061M	ChemiScreen™ $CysLT_1$ Leukotriene family receptor membrane prep
HTS061RTA	Ready-to-Assay™ $CysLT_1$ Leukotriene receptor frozen cells
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

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