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PRODUCT DATASHEET

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

CATALOG NUMBER: HTS061RTA

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 cysteinyl leukotrienes, leukotriene C_4, leukotriene D_4 and leukotriene E_4, 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, $CysLT_1$ and $CysLT_2$. A $CysLT_1$ selective antagonist, montelukast, is used clinically in the treatment of asthma (Brink *et al*., 2003; Evans, 2002). Cloned human $CysLT_1$expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $CysLT_1$ expression on the cell surface for functional detection via the calcium signaling pathway Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at $CysLT_1$.

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

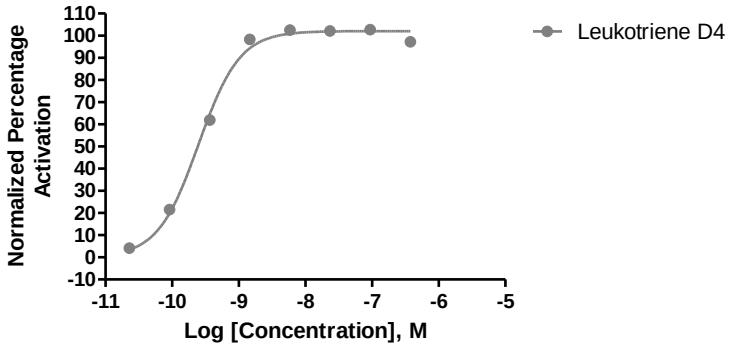

Figure 1. Representative data for activation of $CysLT_1$ receptor. Calcium flux in $CysLT_1$ expressing Chem-1 cell line induced by Leukotriene D4. $CysLT_1$expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 5,500 RLU (Relative Light Units).

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

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Leukotriene D4	Calcium Flux	0.06	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
Leukotriene D4 ligand	Cayman: 20310
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

CYSLTR1 cDNA (Accession Number: NM_006639; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary pHS plasmid.

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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 AAT CAA GTG TAT TCC ACC TTG TAC TCT ATG ATC TCT GTT GTA GGC TTC TTT GGC AAT GGC TTT
GTG CTC TAT GTC CTC ATA AAA ACC TAT CAC AAG AAG TCA GCC TTC CAA GTA TAC ATG ATT AAT TTA GCA GTA GCA GAT CTA
CTT TGT GTG TGC ACA CTG CCT CTC CGT GTG GTC TAT TAT GTT CAC AAA GGC ATT TGG CTC TTT GGT GAC TTC TTG TGC CGC
CTC AGC ACC TAT GCT TTG TAT GTC AAC CTC TAT TGT AGC ATC TTC TTT ATG ACA GCC ATG AGC TTT TTC CGG TGC ATT GCA
ATT GTT TTT CCA GTC CAG AAC ATT AAT TTG GTT ACA CAG AAA AAA GCC AGG TTT GTG TGT GTA GGT ATT TGG ATT TTT GTG
ATT TTG ACC AGT TCT CCA TTT CTA ATG GCC AAA CCA CAA AAA GAT GAG AAA AAT AAT ACC AAG TGC TTT GAG CCC CCA CAA
GAC AAT CAA ACT AAA AAT CAT GTT TTG GTC TTG CAT TAT GTG TCA TTG TTT GTT GGC TTT ATC ATC CCT TTT GTT ATT ATA
ATT GTC TGT TAC ACA ATG ATC ATT TTG ACC TTA CTA AAA AAA TCA ATG AAA AAA AAT CTG TCA AGT CAT AAA AAG GCT ATA
GGA ATG ATC ATG GTC GTG ACC GCT GCC TTT TTA GTC AGT TTC ATG CCA TAT CAT ATT CAA CGT ACC ATT CAC CTT CAT TTT
TTA CAC AAT GAA ACT AAA CCC TGT GAT TCT GTC CTT AGA ATG CAG AAG TCC GTG GTC ATA ACC TTG TCT CTG GCT GCA TCC
AAT TGT TGC TTT GAC CCT CTC CTA TAT TTC TTT TCT GGG GGT AAC TTT AGG AAA AGG CTG TCT ACA TTC AGA AAG CAT TCT
TTG TCC AGC GTG ACT TAT GTA CCC AGA AAG AAG GCC TCT TTG CCA GAA AAA GGA GAA GAA ATA TGT AAA GTA TGA
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS061C	ChemiScreen™ $CysLT_1$ Leukotriene Receptor Stable Cell Line
HTS061M	ChemiScreen™ $CysLT_1$ Leukotriene Receptor membrane prep
HTS061L	ChemiBrite™ $CysLT_1$ Leukotriene Receptor Stable Cell Line

REFERENCES

1. Brink C *et al.* (2003) International Union of Pharmacology XXXVII. Nomenclature for Leukotriene and Lipoxin Receptors. *Pharmacol. Rev.* 55: 195-227.
2. Evans JF (2002) Cysteinyl leukotriene receptors. *Prostaglandins Other Lipid Mediat.* 68-69:587-97.

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