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PRODUCT DATASHEET

ChemiScreen™ $CysLT_2$ Leukotriene Receptor Stable Cell Line

CATALOG NUMBER: HTS088C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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 $CysLT_2$. $CysLT_2$ is expressed in moncytes/macrophages, mast cells and eosinophils. (Brink *et al*., 2003; Kanaoka and Boyce, 2004). A coding polymorphism in $CysLT_2$ that results in a receptor with reduced response to LTD_4 is associated with asthma (Pillai *et al*., 2004). The cloned human $CysLT_2$-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $CysLT_2$ 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 antagonists of interactions between $CysLT_2$ and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay

APPLICATION DATA

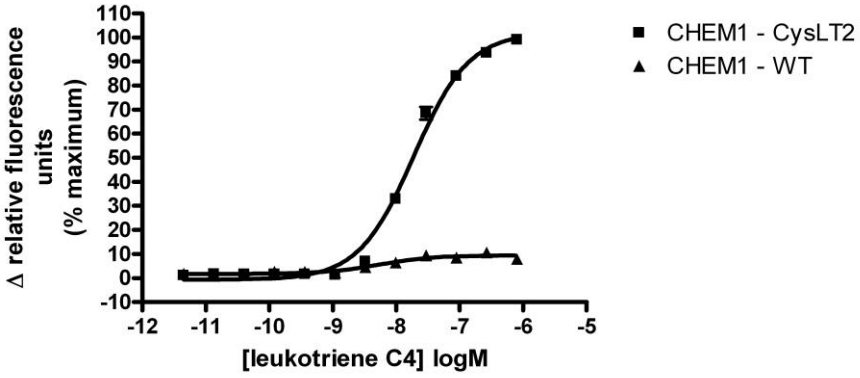

Figure 1. Representative data for activation of $CysLT_2$ receptor stably expressed in Chem-1 cells induced by Leukotriene C_4 using a fluorescent calcium flux assay. $CysLT_2$–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. 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 9,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of $CysLT_2$-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Leukotriene C_4	Calcium Flux - Fluorescence	19	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

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. 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
Leukotriene C_4 ligand	Cayman: 20210
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 – 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

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human $CysLT_2$ cDNA (Accession Number: NM_020377; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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                                                ATG GAG AGA AAA TTT ATG TCC TTG CAA CCA TCC ATC
                                                  M   E   R   K   F   M   S   L   Q   P   S   I

TCC GTA TCA GAA ATG GAA CCA AAT GGC ACC TTC AGC AAT AAC AAC AGC AGG AAC TGC ACA ATT GAA AAC
 S   V   S   E   M   E   P   N   G   T   F   S   N   N   N   S   R   N   C   T   I   E   N

TTC AAG AGA GAA TTT TTC CCA ATT GTA TAT CTG ATA ATA TTT TTC TGG GGA GTC TTG GGA AAT GGG TTG
 F   K   R   E   F   F   P   I   V   Y   L   I   I   F   F   W   G   V   L   G   N   G   L

TCC ATA TAT GTT TTC CTG CAG CCT TAT AAG AAG TCC ACA TCT GTG AAC GTT TTC ATG CTA AAT CTG GCC
 S   I   Y   V   F   L   Q   P   Y   K   K   S   T   S   V   N   V   F   M   L   N   L   A

ATT TCA GAT CTC CTG TTC ATA AGC ACG CTT CCC TTC AGG GCT GAC TAT TAT CTT AGA GGC TCC AAT TGG
 I   S   D   L   L   F   I   S   T   L   P   F   R   A   D   Y   Y   L   R   G   S   N   W

ATA TTT GGA GAC CTG GCC TGC AGG ATT ATG TCT TAT TCC TTG TAT GTC AAC ATG TAC AGC AGT ATT TAT
 I   F   G   D   L   A   C   R   I   M   S   Y   S   L   Y   V   N   M   Y   S   S   I   Y

TTC CTG ACC GTG CTG AGT GTT GTG CGT TTC CTG GCA ATG GTT CAC CCC TTT CGG CTT CTG CAT GTC ACC
 F   L   T   V   L   S   V   V   R   F   L   A   M   V   H   P   F   R   L   L   H   V   T

AGC ATC AGG AGT GCC TGG ATC CTC TGT GGG ATC ATA TGG ATC CTT ATC ATG GCT TCC TCA ATA ATG CTC
 S   I   R   S   A   W   I   L   C   G   I   I   W   I   L   I   M   A   S   S   I   M   L

CTG GAC AGT GGC TCT GAG CAG AAC GGC AGT GTC ACA TCA TGC TTA GAG CTG AAT CTC TAT AAA ATT GCT
 L   D   S   G   S   E   Q   N   G   S   V   T   S   C   L   E   L   N   L   Y   K   I   A

AAG CTG CAG ACC ATG AAC TAT ATT GCC TTG GTG GTG GGC TGC CTG CTG CCA TTT TTC ACA CTC AGC ATC
 K   L   Q   T   M   N   Y   I   A   L   V   V   G   C   L   L   P   F   F   T   L   S   I

TGT TAT CTG CTG ATC ATT CGG GTT CTG TTA AAA GTG GAG GTC CCA GAA TCG GGG CTG CGG GTT TCT CAC
 C   Y   L   L   I   I   R   V   L   L   K   V   E   V   P   E   S   G   L   R   V   S   H

AGG AAG GCA CTG ACC ACC ATC ATC ATC ACC TTG ATC ATC TTC TTC TTG TGT TTC CTG CCC TAT CAC ACA
 R   K   A   L   T   T   I   I   I   T   L   I   I   F   F   L   C   F   L   P   Y   H   T

CTG AGG ACC GTC CAC TTG ACG ACA TGG AAA GTG GGT TTA TGC AAA GAC AGA CTG CAT AAA GCT TTG GTT
 L   R   T   V   H   L   T   T   W   K   V   G   L   C   K   D   R   L   H   K   A   L   V

ATC ACA CTG GCC TTG GCA GCA GCC AAT GCC TGC TTC AAT CCT CTG CTC TAT TAC TTT GCT GGG GAG AAT
 I   T   L   A   L   A   A   A   N   A   C   F   N   P   L   L   Y   Y   F   A   G   E   N

TTT AAG GAC AGA CTA AAG TCT GCA CTC AGA AAA GGC CAT CCA CAG AAG GCA AAG ACA AAG TGT GTT TTC
 F   K   D   R   L   K   S   A   L   R   K   G   H   P   Q   K   A   K   T   K   C   V   F

CCT GTT AGT GTG TGG TTG AGA AAG GAA ACA AGA GTA TAA TGA
 P   V   S   V   W   L   R   K   E   T   R   V     Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS088M	ChemiScreen™ $CysLT_2$ Leukotriene Receptor Membrane Prep

REFERENCES

1. Brink C *et al.* (2003) International Union of Pharmacology XXXVII. Nomenclature for Leukotriene and Lipoxin Receptors. *Pharmacol. Rev.* 55: 195-227.
2. Kanaoka Y and Boyce JA (2004). Cysteinyl Leukotrienes and their receptors: cellular distribution and function in immune and inflammatory responses. *J. Immunol.* 173: 1503-1510.
3. Pillai SG *et al.* (2004) A coding polymorphism in the CYSLT2 receptor with reduced affinity to LTD4 is associated with asthma. *Pharmacogenetics* 14: 627-33.

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