eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ ChemR23 Chemoattractant Receptor Stable Cell Line

CATALOG NUMBER: HTS071C

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.

ChemR23 was discovered as an orphan receptor related to the chemoattractant receptors C3a, C5a and FPR1, and expressed on dendritic cells and macrophages (Samson *et al.*, 1998). A ligand for ChemR23 was characterized as chemerin, a 15 kD proteolytically processed protein found in inflammatory sites; a 9 amino acid peptide from the C-terminus of chemerin is sufficient to activate ChemR23 (Wittamer *et al.*, 2003, 2004). Chemerin expressed in lymphoid and microvascular endothelium mediates migration of ChemR23-expressing dendritic cells to lymphoid organs and vasculature at sites of inflammation (Vermi *et al.*, 2005). In addition, a bioactive lipid, resolvin E1, was found to functionally interact with ChemR23 to reduce inflammation (Arita et al., 2005). The cloned human ChemR23-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant ChemR23 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 ChemR23 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

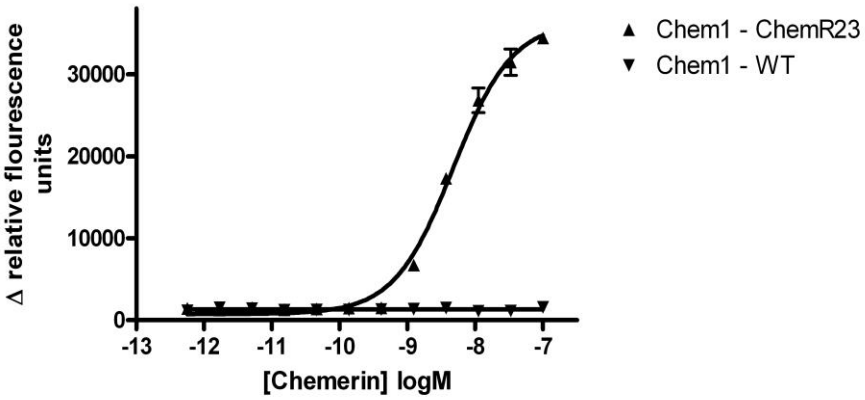

Figure 1. Representative data for activation of ChemR23 receptor stably expressed in Chem-1 cells induced by Chemerin using a fluorescent calcium flux assay. ChemR23–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of ChemR23-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Chemerin	Calcium Flux - Fluorescence	4.7	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
Chemerin ligand	R&D Systems: 2324-CM
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 ChemR23 cDNA (Accession Number: NM_004072; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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                                                  ATG GAG GAT GAA GAT TAC AAC ACT

TCC ATC AGT TAC GGT GAT GAA TAC CCT GAT TAT TTA GAC TCC ATT GTG GTT TTG GAG GAC

TTA TCC CCC TTG GAA GCC AGG GTG ACC AGG ATC TTC CTG GTG GTG GTC TAC AGC ATC GTC

TGC TTC CTC GGG ATT CTG GGC AAT GGT CTG GTG ATC ATC ATT GCC ACC TTC AAG ATG AAG

AAG ACA GTG AAC ATG GTC TGG TTC CTC AAC CTG GCA GTG GCA GAT TTC CTG TTC AAC GTC

TTC CTC CCA ATC CAT ATC ACC TAT GCC GCC ATG GAC TAC CAC TGG GTT TTC GGG ACA GCC

ATG TGC AAG ATC AGC AAC TTC CTT CTC ATC CAC AAC ATG TTC ACC AGC GTC TTC CTG CTG

ACC ATC ATC AGC TCT GAC CGC TGC ATC TCT GTG CTC CTC CCT GTC TGG TCC CAG AAC CAC

CGC AGC GTT CGC CTG GCT TAC ATG GCC TGC ATG GTC ATC TGG GTC CTG GCT TTC TTC TTG

AGT TCC CCA TCT CTC GTC TTC CGG GAC ACA GCC AAC CTG CAT GGG AAA ATA TCC TGC TTC

AAC AAC TTC AGC CTG TCC ACA CCT GGG TCT TCC TCG TGG CCC ACT CAC TCC CAA ATG GAC

CCT GTG GGG TAT AGC CGG CAC ATG GTG GTG ACT GTC ACC CGC TTC CTC TGT GGC TTC CTG

GTC CCA GTC CTC ATC ATC ACA GCT TGC TAC CTC ACC ATC GTC TGC AAA CTG CAG CGC AAC

CGC CTG GCC AAG ACC AAG AAG CCC TTC AAG ATT ATT GTG ACC ATC ATC ATT ACC TTC TTC

CTC TGC TGG TGC CCC TAC CAC ACA CTC AAC CTC CTA GAG CTC CAC CAC ACT GCC ATG CCT

GGC TCT GTC TTC AGC CTG GGT TTG CCC CTG GCC ACT GCC CTT GCC ATT GCC AAC AGC TGC

ATG AAC CCC ATT CTG TAT GTT TTC ATG GGT CAG GAC TTC AAG AAG TTC AAG GTG GCC CTC

TTC TCT CGC CTG GTC AAT GCT CTA AGT GAA GAT ACA GGC CAC TCT TCC TAC CCC AGC CAT

AGA AGC TTT ACC AAG ATG TCA TCA ATG AAT GAG AGG ACT TCT ATG AAT GAG AGG GAG ACC

GGC ATG CTT TGA
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS071M	ChemiScreen™ ChemR23 Chemoattractant Receptor Membrane Prep

REFERENCES

1. Arita M *et al.* (2005) Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* 201: 713-22.
2. Samson M *et al.* (1998) ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* 28: 1689-700.
3. Vermi W *et al.* (2005) Role of ChemR23 in directing the migration of myeloid and plasmacytoid dendritic cells to lymphoid organs and inflamed skin. *J. Exp. Med.* 201:509-15.
4. Wittamer *et al.* (2003) Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *J. Exp. Med.* 198: 977-985.
5. Wittamer *et al.* (2004) The C-terminal nonapeptide of mature chemerin activates the chemerin receptor with low nanomolar potency. *J. Biol. Chem.* 279: 9956-9962.

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