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PRODUCT DATASHEET

ChemiScreen™ CXCR5 Chemokine Receptor Stable Cell Line

CATALOG NUMBER: HTS055C

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 Chem-10 host is derived from Chem-1.

CXCR5 (also known as BLR1) is a GPCR that binds selectively to the chemokine CXCL13 (also known as BLC and BCA-1) to mediate immune system development and function (Förster *et al.*, 1996; Gunn *et al.*, 1998). CXCR5 cooperates with CCR7 to determine functional organization of lymphoid organs such as lymph nodes and Peyer's patches (Ohl *et al.*, 2003). In addition, CXCR5 mediates migration of B cells into the follicles of the splenic white pulp, thus permitting antigen deposition on follicular dendritic cells (Förster *et al.*, 1996; Cinamon *et al.*, 2008). Patients with rheumatoid arthritis (RA) displayed upregulated expression of CXCR5 to a greater extent than other chemokine receptors, and CXCR5-null mice subjected to antigen-induced arthritis display reduced joint destruction (Schmutz et al, 2004; Wengner et al, 2007). Therefore, CXCR5 represents a potential target for treatment of rheumatoid arthritis. Cloned human CXCR5-expressing cell line is made in the Chem-10 host, which supports high levels of recombinant CXCR5 expression on the cell surface and contains optimal levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at CXCR5.

USE RESTRICTIONS

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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.

APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

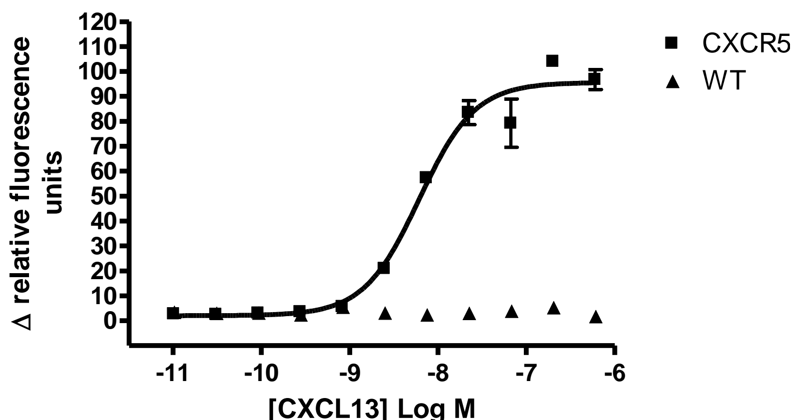

Figure 1. Representative data for activation of CXCR5 receptor stably expressed in Chem-1 cells induced by CXCL13 using a fluorescent calcium flux assay. CXCR5–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 13,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of CXCR5-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
CXCL13	Calcium Flux - Fluorescence	6.8	Eurofins Internal Data
CXCL13	Calcium Flux - Fluorescence	100	Gunn *et al.*, 1998

* 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
	Hygromycin	250 µg/ml	Invivogen: ant-hg-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

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
CXCL5 ligand	R&D Systems: 801-CX
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 5×10^5 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.

Eurofins Discovery Services

HOST CELL

Chem-10, a rat adherent cell line of hematopoietic origin expressing a recombinant promiscuous G-protein.

EXOGENOUS GENE EXPRESSION

Human CXCR5 cDNA (Accession Number: NM_0001716; see CODING SEQUENCE below)

CODING SEQUENCE

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   1 - ATG AAC TAC CCG CTA ACG CTG GAA ATG GAC CTC GAG AAC CTG GAG GAC CTG TTC TGG GAA CTG GAC AGA TTG  - 72
   1 -  M   N   Y   P   L   T   L   E   M   D   L   E   N   L   E   D   L   F   W   E   L   D   R   L   - 24

  73 - GAC AAC TAT AAC GAC ACC TCC CTG GTG GAA AAT CAT CTC TGC CCT GCC ACA GAG GGG CCC CTC ATG GCC TCC  - 144
  25 -  D   N   Y   N   D   T   S   L   V   E   N   H   L   C   P   A   T   E   G   P   L   M   A   S   - 48

 145 - TTC AAG GCC GTG TTC GTG CCC GTG GCC TAC AGC CTC ATC TTC CTC CTG GGC GTG ATC GGC AAC GTC CTG GTG  - 216
  49 -  F   K   A   V   F   V   P   V   A   Y   S   L   I   F   L   L   G   V   I   G   N   V   L   V   - 72

 217 - CTG GTG ATC CTG GAG CGG CAC CGG CAG ACA CGC AGT TCC ACG GAG ACC TTC CTG TTC CAC CTG GCC GTG GCC  - 288
  73 -  L   V   I   L   E   R   H   R   Q   T   R   S   S   T   E   T   F   L   F   H   L   A   V   A   - 96

 289 - GAC CTC CTG CTG GTC TTC ATC TTG CCC TTT GCC GTG GCC GAG GGC TCT GTG GGC TGG GTC CTG GGG ACC TTC  - 360
  97 -  D   L   L   L   V   F   I   L   P   F   A   V   A   E   G   S   V   G   W   V   L   G   T   F   - 120

 361 - CTC TGC AAA ACT GTG ATT GCC CTG CAC AAA GTC AAC TTC TAC TGC AGC AGC CTG CTC CTG GCC TGC ATC GCC  - 432
 121 -  L   C   K   T   V   I   A   L   H   K   V   N   F   Y   C   S   S   L   L   L   A   C   I   A   - 144

 433 - GTG GAC CGC TAC CTG GCC ATT GTC CAC GCC GTC CAT GCC TAC CGC CAC CGC CGC CTC CTC TCC ATC CAC ATC  - 504
 145 -  V   D   R   Y   L   A   I   V   H   A   V   H   A  Y   R   H   R   R   L   L   S   I   H   I   - 168

 505 - ACC TGT GGG ACC ATC TGG CTG GTG GGC TTC CTC CTT GCC TTG CCA GAG ATT CTC TTC GCC AAA GTC AGC CAA  - 576
 169 -  T   C   G   T   I   W   L   V   G   F   L   L   A   L   P   E   I   L   F   A   K   V   S   Q   - 192

 577 - GGC CAT CAC AAC AAC TCC CTG CCA CGT TGC ACC TTC TCC CAA GAG AAC CAA GCA GAA ACG CAT GCC TGG TTC  - 648
 193 -  G   H   H   N   N   S   L   P   R   C   T   F   S   Q   E   N   Q   A   E   T   H   A   W   F   - 216

 649 - ACC TCC CGA TTC CTC TAC CAT GTG GCG GGA TTC CTG CTG CCC ATG CTG GTG ATG GGC TGG TGC TAC GTG GGG  - 720
 217 -  T   S   R   F   L   Y   H   V   A   G   F   L   L   P   M   L   V   M   G   W   C   Y   V   G   - 240

 721 - GTA GTG CAC AGG TTG CGC CAG GCC CAG CGG CGC CCT CAG CGG CAG AAG GCA GTC AGG GTG GCC ATC CTG GTG  - 792
 241 -  V   V   H   R   L   R   Q   A   Q   R   R   P   Q   R   Q   K   A   V   R   V   A   I   L   V   - 264

 793 - ACA AGC ATC TTC TTC CTC TGC TGG TCA CCC TAC CAC ATC GTC ATC TTC CTG GAC ACC CTG GCG AGG CTG AGG  - 864
 265 -  T   S   I   F   F   L   C   W   S   P   Y   H   I   V   I   F   L   D   T   L   A   R   L   K   - 288

 865 - GCC GTG GAC AAT ACC TGC AAG CTG AAT GGC TCT CTC CCC GTG GCC ATC ACC ATG TGT GAG TTC CTG GGC CTG  - 936
 289 -  A   V   D   N   T   C   K   L   N   G   S   L   P   V   A   I   T   M   C   E   F   L   G   L   - 312

 937 - GCC CAC TGC TGC CTC AAC CCC ATG CTC TAC ACT TTC GCC GGC GTG AAG TTC CGC AGT GAC CTG TCG CGG CTC  - 1008
 313 -  A   H   C   C   L   N   P   M   L   Y   T   F   A   G   V   K   F   R   S   D   L   S   R   L   - 336

1009 - CTG ACG AAG CTG GGC TGT ACC GGC CCT GCC TCC CTG TGC CAG CTC TTC CCT AGC TGG CGC AGG AGC AGT CTC  - 1080
 337 -  L   T  K   L   G   C   T   G   P   A   S   L   C   Q   L   F   P   S   W   R   R   S   S   L  - 360

1081 - TCT GAG TCA GAG AAT GCC ACC TCT CTC ACC ACG TTC TGA
 361 -  S   E   S   E   N   A   T   S   L   T   T   F  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS055M	ChemiScreen™ CXCR5 Chemokine family receptor membrane prep

REFERENCES

1. Cinamon G *et al.* (2008) Follicular shuttling of marginal zone B cells facilitates antigen transport. Nat. Immunol. 9: 54-62.
2. Gunn MD *et al.* (1998) A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. Nature 391: 799-803.
3. Ohl L *et al.* (2003) Cooperating mechanisms of CXCR5 and CCR7 in development and organization of secondary lymphoid organs. J. Exp. Med. 197: 1199-1204
4. Schmutz C *et al.* (2004) Chemokine receptors in the rheumatoid synovium: upregulation of CXCR5. Arthritis Res. Ther. 7: R217-R229
5. Wengner AM *et al.* (2007) CXCR5- and CCR7-dependent lymphoid neogenesis in a murine model of chronic antigen-induced arthritis. Arthritis Rheum. 56: 3271-3283.

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