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PRODUCT DATASHEET

ChemiScreen™ CXCR6 Chemokine Receptor Stable Cell Line

CATALOG NUMBER: HTS054C

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-5 host, which supports high levels of functional receptor expression on the cell surface. Chem-5 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

The GPCR CXCR6 (previously known as BONZO, STRL33 and TYMSTR) binds selectively to the free chemokine domain of CXCL16, which is derived from a membrane-bound precursor containing a CXC-containing chemokine domain, a glycosylated mucin-like domain, and a transmembrane domain (Wilbanks *et al*., 2001). CXCR6 is selectively expressed on Th1, Th2 and Tr1 T cell subsets, whereas CXCL16 is expressed on monocytes/macrophages and dendritic cells (Tabata *et al*., 2005). CXCR6 functions as a cofactor with CD4 for HIV entry and Env-mediated cell fusion (Liao *et al*., 1997). Binding of CXCL16 to CXCR6 promotes migration of activated lymphocytes to sites of inflammation in tissues such as liver and synovium (Nanki *et al.*, 2005, Sato *et al.*, 2005). The cloned human CXCR6 -expressing cell line is made in the Chem-5 host, which supports high levels of recombinant CXCR6 expression on the cell surface and contains high levels of the 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 of CXCR6.

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

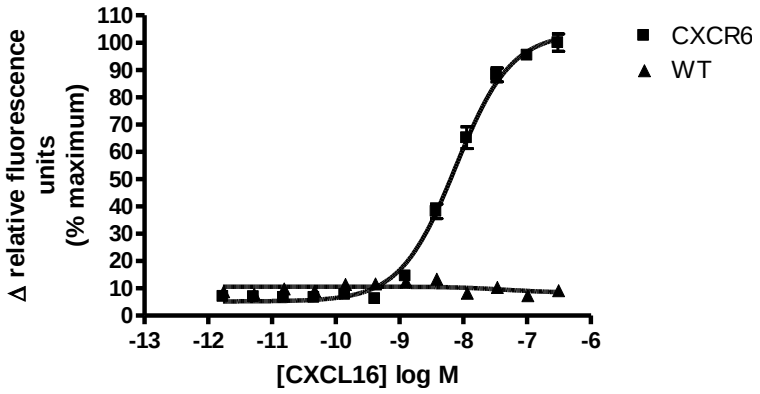

Figure 1. Representative data for activation of CXCR6 receptor stably expressed in Chem-5 cells induced by CXCL16 using a fluorescent calcium flux assay. CXCR6–expressing Chem-5 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-5) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of CXCR6-expressing Chem-5 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
CXCL16	Calcium Flux - Fluorescence	7.5	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. The Z' value, as defined with response to 10µM 2MeSATP, was 0.83.

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

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
CXCL16 ligand	Peprotech: 300-55
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-5, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human CXCR6 cDNA (Accession Number: NM_006564; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

```
   1 - ATG GCA GAG CAT GAT TAC CAT GAA GAC TAT GGG TTC AGC AGT TTC AAT GAC AGC AGC CAG GAG GAG CAT CAA
   1 -  M   A   E   H   D   Y   H   E   D   Y   G   F   S   S   F   N   D   S   S   Q   E   E   H   Q
  73 - GAC TTC CTG CAG TTC AGC AAG GTC TTT CTG CCC TGC ATG TAC CTG GTG GTG TTT GTC TGT GGT CTG GTG GGG
  25 -  D   F   L   Q   F   S   K   V   F   L   P   C   M   Y   L   V   V   F   V   C   G   L   V   G
 145 - AAC TCT CTG GTG CTG GTC ATA TCC ATC TTC TAC CAT AAG TTG CAG AGC CTG ACG GAT GTG TTC CTG GTG AAC
  49 -  N   S   L   V   L   V   I   S   I   F   Y   H   K   L   Q   S   L   T   D   V   F   L   V   N
 217 - CTA CCC CTG GCT GAC CTG GTG TTT GTC TGC ACT CTG CCC TTC TGG GCC TAT GCA GGC ATC CAT GAA TGG GTG
  73 -  L   P   L   A   D   L   V   F   V   C   T   L   P   F   W   A   Y   A   G   I   H   E   W   V
 289 - TTT GGC CAG GTC ATG TGC AAG AGC CTA CTG GGC ATC TAC ACT ATT AAC TTC TAC ACG TCC ATG CTC ATC CTC
  97 -  F   G   Q   V   M   C   K   S   L   L   G   I   Y   T   I   N   F   Y   T   S   M   L   I   L
 361 - ACC TGC ATC ACT GTG GAT CGT TTC ATT GTA GTG GTT AAG GCC ACC AAG GCC TAC AAC CAG CAA GCC AAG AGG
 121 -  T   C   I   T   V   D   R   F   I   V   V   V   K   A   T   K   A   Y   N   Q   Q   A   K   R
 433 - ATG ACC TGG GGC AAG GTC ACC AGC TTG CTC ATC TGG GTG ATA TCC CTG CTG GTT TCC TTG CCC CAA ATT ATC
 145 -  M   T   W   G   K   V   T   S   L   L   I   W   V   I   S   L   L   V   S   L   P   Q   I   I
 505 - TAT GGC AAT GTC TTT AAT CTC GAC AAG CTC ATA TGT GGT TAC CAT GAC GAG GCA ATT TCC ACT GTG GTT CTT
 169 -  Y   G   N   V   F   N   L   D   K   L   I   C   G   Y   H   D   E   A   I   S   T   V   V   L
 577 - GCC ACC CAG ATG ACA CTG GGG TTC TTC TTG CCA CTG CTC ACC ATG ATT GTC TGC TAT TCA GTC ATA ATC AAA
 193 -  A   T   Q   M   T   L   G   F   F   L   P   L   L   T   M   I   V   C   Y   S   V   I   I   K
 649 - ACA CTG CTT CAT GCT GGA GGC TTC CAG AAG CAC AGA TCT CTA AAG ATC ATC TTC CTG GTG ATG GCT GTG TTC
 217 -  T   L   L   H   A   G   G   F   Q   K   H   R   S   L   K   I   I   F   L   V   M   A   V   F
 721 - CTG CTG ACC CAG ATG CCC TTC AAC CTC ATG AAG TTC ATC CGC AGC ACA CAC TGG GAA TAC TAT GCC ATG ACC
 241 -  L   L   T   Q   M   P   F   N   L   M   K   F   I   R   S   T   H   W   E   Y   Y   A   M   T
 793 - AGC TTT CAC TAC ACC ATC ATG GTG ACA GAG GCC ATC GCA TAC CTG AGG GCC TGC CTT AAC CCT GTG CTC TAT
 265 -  S   F   H   Y   T   I   M   V   T   E   A   I   A   Y   L   R   A   C   L   N   P   V   L   Y
 865 - GCC TTT GTC AGC CTG AAG TTT CGA AAG AAC TTC TGG AAA CTT GTG AAG GAC ATT GGT TGC CTC CCT TAC CTT
 289 -  A   F   V   S   L   K   F   R   K   N   F   W   K   L   V   K   D   I   G   C   L   P   Y   L
 937 - GGG GTC TCA CAT CAA TGG AAA TCT TCT GAG GAC AAT TCC AAG ACT TTT TCT GCC TCC CAC AAT GTG GAG GCC
 313 -  G   V   S   H   Q   W   K   S   S   E   D   N   S   K   T   F   S   A   S   H   N   V   E   A
1009 - ACC AGC ATG TTC CAG TTA TGA
 337 -  T   S   M   F   Q   L  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-5	ChemiScreen™ Chem-5 Parental Cell Line (control cells)
HTS054M	ChemiScreen™ CXCR6 Chemokine Receptor Membrane Prep

REFERENCES

1. Liao F *et al.* (1997) STRL33, A novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* 185: 2015-23.
2. Nanki T *et al*. (2005) Pathogenic role of the CXCL16-CXCR6 pathway in rheumatoid arthritis. *Arthritis Rheum*. 52: 3004-3014.
3. Sato T *et al*. (2005) Role for CXCR6 in recruitment of activated CD8+ lymphocytes to inflamed liver. *J. Immunol.* 174: 277-283.
4. Tabata S *et al.* (2005) Distribution and kinetics of SR-PSOX/CXCL16 and CXCR6 expression on human dendritic cell subsets and CD4+ T cells. *J. Leukoc. Biol.* 77: 777-786.
5. Wilbanks A *et al.* (2001) Expression cloning of the STRL33/BONZO/TYMSTR ligand reveals elements of CC, CXC, and CX3C chemokines. *J. Immunol.* 166: 5145-5154.

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