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PRODUCT DATASHEET

ChemiBrite CXCR3 Chemokine Receptor Stable Cell Line

CATALOG NUMBER: HTS003L

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

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiBrite cell lines are constructed in the HEK293 host, which supports high levels of functional receptor expression on the cell surface. HEK293 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

CXCR3 is a 7-TM GPCR that is selective for the CXC chemokines IP10, I-TAC and MIG (Loetscher *et al.*, 1996). Binding of IP10 and MIG to CXCR3 induces Ca2+ mobilization, chemotaxis and inflammatory responses of T lymphocytes, and also act as potent inhibitors of angiogenesis. CXCR3 is highly expressed in IL-2-activated T lymphocytes in vitro (Loetscher *et al.,* 1996), and in T lymphocytes present in inflamed tissues in rheumatoid arthritis and multiple sclerosis (Balashoy *et al.*, 1999; Qin *et al.*, 1998). In vivo, neutralization of CXCR3 inhibits experimentally induced type I diabetes (Frigerio *et al.*, 2002), peritonitis (Xie *et al.*, 2003), and post-lung transplantation bronchiolitis obliterans syndrome (Belperio *et al.*, 2002). Cloned CXCR3 receptor-expressing ChemiBrite cells were constructed by stable transfection of HEK293 cells with ChemiBrite clytin, the receptor and a promiscuous G protein to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the CXCR3 receptor.

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GMO

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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: Luminescent Mode and Fluorescent Mode

APPLICATION DATA

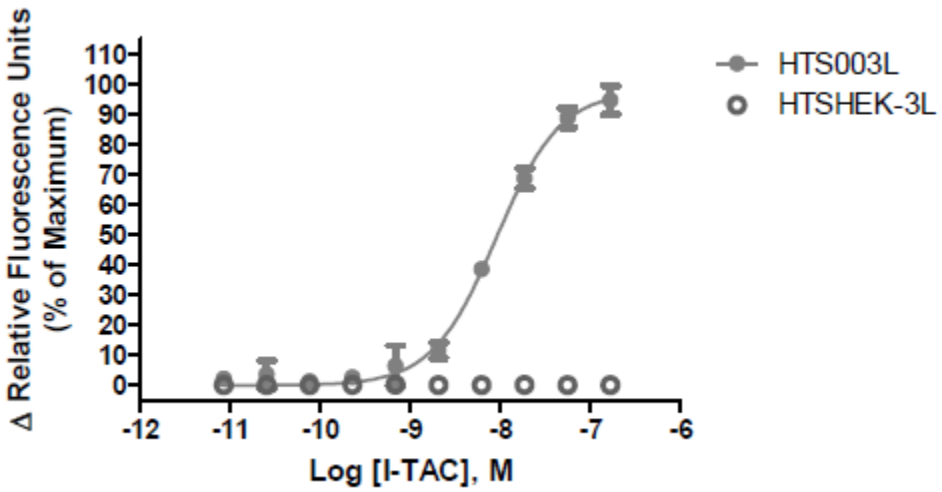

Figure 1. Representative data for activation of the CXCR3 receptor stably expressed in HEK293 cells induced by I-TAC using a fluorescent calcium flux assay. CXCR3–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 12,000 RLU.

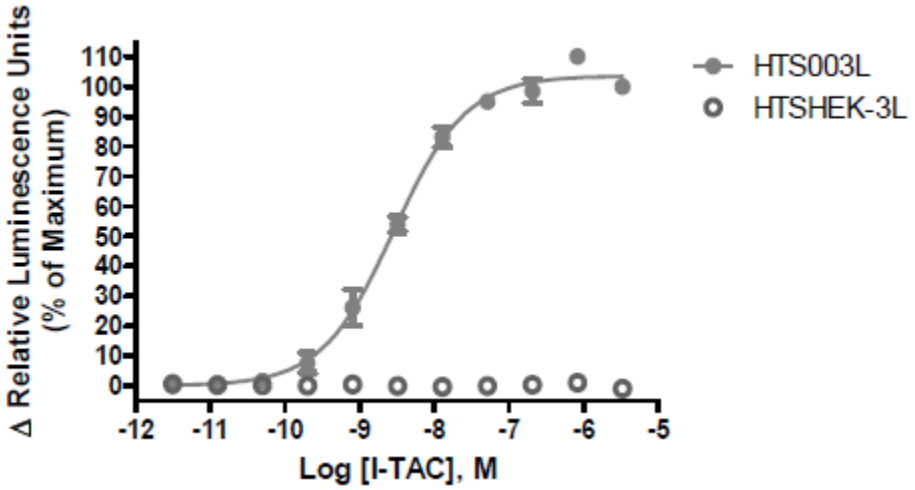

Figure 2. Representative data for activation of CXCR3 receptor stably expressed in HEK293 cells induced by I-TAC using a luminescent calcium flux assay. CXCR3 –expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand was determined on a Molecular Devices FLIPR$^{TETRA®}$ with ICCD camera in 96-well format with a final concentration of 0.5% DMSO. Luminescence signal obtained in this experiment was 80,000 RLU (Relative Light Units) as measured by AUC (are under curve) for 80 s post agonist addition using the provided protocol. Similarly parental cells (catalog #: HTSHEK-3L) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of CXCR3-expressing HEK293 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
I-TAC	Calcium Flux - Fluorescence	9.0	Eurofins Internal Data
I-TAC	Calcium Flux - Luminescence	2.9	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 166 nM I-TAC, was 0.7.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM/F12 Medium	-	Millipore: DF-041-B
	Dialyzed, Heat Inactivated FBS	10%	Hyclone: SH30079.03
	Non-Essential Amino Acids (NEAA)	1X	Millipore: TMS-001-C
Selection Medium	Basal Medium (see above)	-	
	Puromycin	1 µg/ml	Merck EMD: 400053
	Geneticin (G418)	200 µg/ml	Merck EMD: 345812
	Hygromycin	100 µg/ml	Merck EMD: 540411
Dissociation	Sterile PBS	-	Millipore: BSS-1006A
	0.05% Trypsin-EDTA	-	Millipore: SM-2002-C
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Gibco: 16000
	Dimethyl Sulfoxide (DMSO)	10%	Merck EMD: 317275

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	2.0	24
T75	15	1.5	48
T75	15	1.0	72
T150	30	4.0	24
T150	30	3.0	48
T150	30	2.0	72

ASSAY SETUP

Luminescence

Table 4. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Luminescence
Ex/Em	None/None
Camera Gain	280,000
Gate Open	100 %
Exposure Time	0.9 sec
Read Interval	1 sec.
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
BSA (Protease Free). Prepare to 1% in H2O, filter	Merck EMD: 126609
I-TAC ligand	Peprotech: 300-46
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine (250µg). Prepare to 10mM	Merck EMD: 233900

Assay Protocol – Luminescence

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, 0.1% BSA, pH 7.4) and Loading buffer (Assay buffer with 10µM coelenterazine). *Note: Please prepare coelenterazine stock according to Manufacturer's Recommendations at 10mM to allow for 1:1000 dilution into Loading buffer (10uM final concentration). It is critical that coelenterazine solution is prepared at room temperature and is protected from light.*
7. Remove medium from assay plate by inverting and tapping/flicking plate. Blot plate.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 3 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 4. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. Export data to analyze using the area under the curve statistic.

HOST CELL

HEK293

EXOGENOUS GENE EXPRESSION

Human CXCR3 cDNA (Accession Number: NM_001504.1; see CODING SEQUENCE below) and a proprietary mutant clytin photoprotein and promiscuous G protein expressed in a bicistronic vector

CODING SEQUENCE

```
ATG GTC CTT GAG GTG AGT GAC CAC CAA GTG CTA AAT GAC GCC GAG GTT GCC GCC CTC CTG GAG AAC TTC AGC TCT
 M   V   L   E   V   D   H   Q   V   L   N   D   A   E   V   A   A   L   L   E   N   F   S   S   S

TCC TAT GAC TAT GGA GAA AAC GAG AGT GAC TCG TGC TGT ACC TCC CCG CCC TGC CCA CAG GAC TTC AGC CTG AAC
 S   Y   D   Y   G   E   N   E   S   D   S   C   C   T   S   P   P   C   P   Q   D   F   S   L   N

TTC GAC CGG GCC TTC CTG CCA GCC CTC TAC AGC CTC CTC TTT CTG CTG GGG CTG CTG GGC AAC GGC GCG GTG GCA
 F   D   R   A   F   L   P   A   L   Y   S   L   L   F   L   L   G   L   L   G   N   G   A   V   A

GCC GTG CTG CTG AGC CGG CGG ACA GCC CTG AGC AGC ACC GAC ACC TTC CTG CTC CAC CTA GCT GTA GCA GAC ACG
 A   V   L   L   S   R   R   T   A   L   S   S   T   D   T   F   L   L   H   L   A   V   A   D   T

CTG CTG GTG CTG ACA CTG CCG CTC TGG GCA GTG GAC GCT GCC GTC CAG TGG GTC TTT GGC TCT GGC CTC TGC AAA
 L   L   V   L   T   L   P   L   W   A   V   D   A   A   V   Q   W   V   F   G   S   G   L   C   K

GTG GCA GGT GCC CTC TTC AAC ATC AAC TTC TAC GCA GGA GCC CTC CTG CTG GCC TGC ATC AGC TTT GAC CGC TAC
 V   A   G   A   L   F   N   I   N   F   Y   A   G   A   L   L   L   A   C   I   S   F   D   R   Y
CTG AAC ATA GTT CAT GCC ACC CAG CTC TAC CGC CGG GGG CCC CCG GCC CGC GTG ACC CTC ACC TGC CTG GCT GTC
 L   N   I   V   H   A   T   Q   L   Y   R   R   G   P   P   A   R   V   T   L   T   C   L   A   V

TGG GGG CTC TGC CTG CTT TTC GCC CTC CCA GAC TTC ATC TTC CTG TCG GCC CAC CAC GAC GAG CGC CTC AAC GCC
 W   G   L   C   L   L   F   A   L   P   D   F   I   F   L   S   A   H   H   D   E   R   L   N   A

ACC CAC TGC CAA TAC AAC TTC CCA CAG GTG GGC CGC ACG GCT CTG CGG GTG CTG CAG CTG GTG GCT GGC TTT CTG
 T   H   C   Q   Y   N   F   P   Q   V   G   R   T   A   L   R   V   L   Q   L   V   A   G   F   L

CTG CCC CTG CTG GTC ATG GCC TAC TGC TAT GCC CAC ATC CTG GCC GTG CTG CTG GTT TCC AGG GGC CAG CGG CGC
 L   P   L   L   V   M   A   Y   C   Y   A   H   I   L   A   V   L   L   V   S   R   G   Q   R   R

CTG CGG GCC ATG CGG CTG GTG GTG GTG GTC GTG GTG GCC TTT GCC CTC TGC TGG ACC CCC TAT CAC CTG GTG GTG
 L   R   A   M   R   L   V   V   V   V   V   V   A   F   A   L   C   W   T   P   Y   H   L   V   V

CTG GTG GAC ATC CTC ATG GAC CTG GGC GCT TTG GCC CGC AAC TGT GGC CGA GAA AGC AGG GTA GAC GTG GCC AAG
 L   V   D   I   L   M   D   L   G   A   L   A   R   N   C   G   R   E   S   R   V   D   V   A   K

TCG GTC ACC TCA GGC CTG GGC TAC ATG CAC TGC TGC CTC AAC CCG CTG CTC TAT GCC TTT GTA GGG GTC AAG TTC
 S   V   T   S   G   L   G   Y   M   H   C   C   L   N   P   L   L   Y   A   F   V   G   V   K   F

CGG GAG CGG ATG TGG ATG CTG CTC TTG CGC CTG GGC TGC CCC AAC CAG AGA GGG CTC CAG AGG CAG CCA TCG TCT
 R   E   R   M   W   M   L   L   L   R   L   G   C   P   N   Q   R   G   L   Q   R   Q   P   S   S

TCC CGC CGG GAT TCA TCC TGG TCT GAG ACC TCA GAG GCC TCC TAC TCG GGC TTG TGA
 S   R   R   D   S   S   W   S   E   T   S   E   A   S   Y   S   G   L
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RELATED PRODUCTS

Product Number	Description
HTSHEK-3L	ChemiBrite™ HEK293 Parental Stable Cell Line with Gαqi
HTS003M	ChemiScreen™ CXCR3 Chemokine family receptor membrane prep
HTS003RTA	Ready-to-Assay™ CXCR3 Chemokine receptor frozen cells
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. Loetscher M, *et al*. (1996) Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes.*J. Exp. Med.* 184(3): 963-9.
2. Balashov, KE, *et al.* (1999) CCR5 (+) and CXCR3 (+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions.*Proc. Natl. Acad. Sci. USA* 96: 6873-8.
3. Qin, S, *et al.* (1998) The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions.*J. Clin. Invest.* 101: 746-54.
4. Frigerio, S, *et al.* (2002) Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nat. Med.* 8: 1414-20.
5. Xie, JH, *et al.* (2003) Antibody-mediated blockade of the CXCR3 chemokine receptor results in diminished recruitment of T helper 1 cells into sites of inflammation.*J. Leukoc. Biol.* 73: 771-7-80.
6. Belperio, JA, *et al.* (2002) Critical role for CXCR3 chemokine biology in the pathogenesis of bronchiolitis obliterans syndrome. *J. Immunol.* 169: 1037-1049.

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