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PRODUCT DATASHEET

Ready-to-Assay™ ChemiBrite™ CXCR3 Chemokine Receptor Frozen Cells

CATALOG NUMBER: HTS003LRTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

ChemiBrite™ cells express a novel variant of clytin, a calcium-activated photoprotein, to enable sensitive luminescent detection of ligand-induced calcium flux. The ChemiBrite version of clytin contains a mutation that increases its affinity for calcium to a level that permits detection of cytosolic calcium in many cells with greater sensitivity than other photoproteins targeted to the mitochondria. Luminescent calcium assays offer several advantages over fluorescent calcium assays including increased sensitivity and lack of interference from fluorescent compounds.

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.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

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 Assays: Luminescent Mode and Fluorescent Mode

APPLICATION DATA

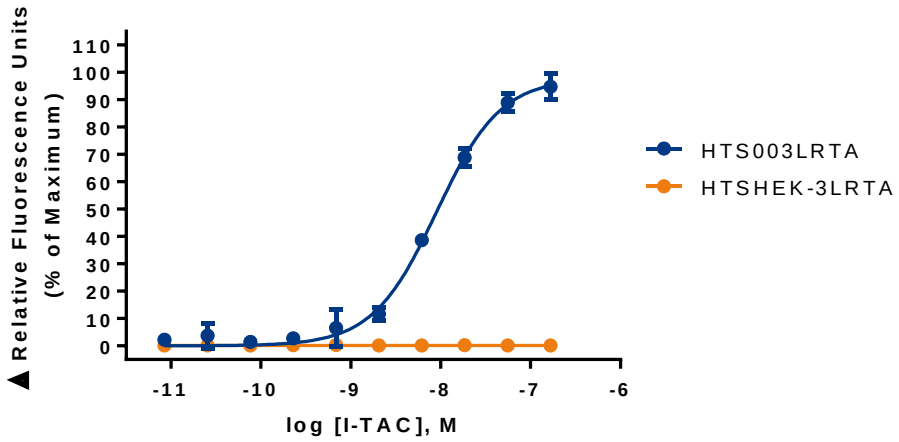

Figure 1. Representative data for activation of the CXCR3 receptor stably expressed in HEK293 cells induced by I-TAC using a fluorescent calcium flux assay. CXCR3–expressing HEK293 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent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

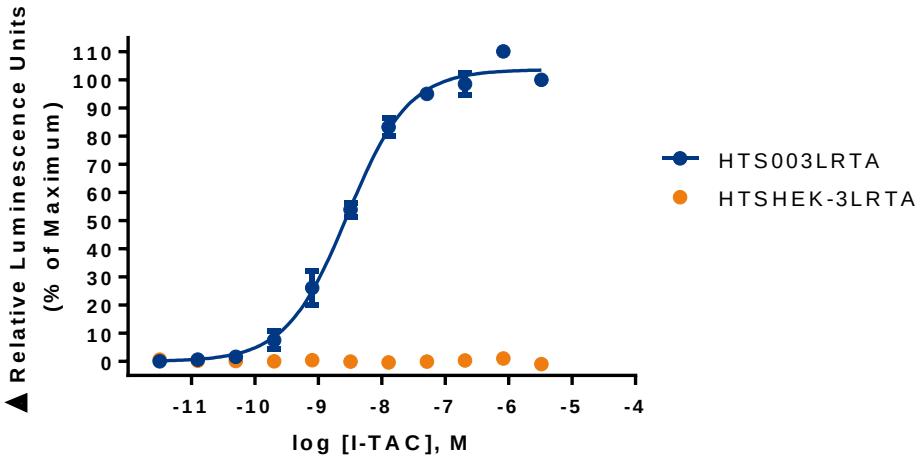

Figure 2. Representative data for activation of CXCR3 receptor stably expressed in HEK293 cells induced by I-TAC using a luminescent calcium flux assay. CXCR3 –expressing HEK293 cells were loaded with 10 µM coelenterazine for 3 h at room temperature and calcium flux in response to the indicated ligand 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 (nM)	REFERENCE
I-TAC	Calcium Flux - Fluorescence	9.0	Eurofins Internal Data
I-TAC	Calcium Flux - Luminescence	2.9	Eurofins Internal Data

ASSAY SETUP

Luminescence

Table 2. 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 3. Luminescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Quest Fluo-8™, AM	AAT Bioquest: 21080
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-h (250µg). Prepare to 10mM	Promega: S2011

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. Fluorescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Probenicid	Sigma: P8761
I-TAC ligand	Peprotech: 300-46
Quest Fluo-8™, AM	AAT Bioquest: 21080
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

Assay Protocol – Luminescence

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
9. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 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.*
10. Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media Component.
11. 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.
12. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
13. 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.
14. After the run is complete, apply subtract bias on sample 1. Export data to analyze using the area under the curve statistic.

Assay Protocol – Fluorescence

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
8. 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
9.	Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media Component.
10.	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.
11.	Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
12.	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.
13.	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

HEK293

EXONGENOUS 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
HTSHEK-3L	Ready-to-Assay™ ChemiBrite™ HEK293 Frozen Cells with Gαqi
HTS003M	ChemiScreen™ CXCR3 Chemokine family receptor membrane prep
HTS003L	ChemiBrite™ CXCR3 Stable Cell Line
HTS003C	ChemiScreen™ CXCR3 Chemokine receptor Stable Cell Line
HTS003RTA	Ready-to-Assay™ CXCR3 Chemokine receptor frozen cells
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells

REFERENCES

a. 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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