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PRODUCT DATASHEET

Ready-to-Assay™ CXCR5 Chemokine Receptor Frozen Cells

CATALOG NUMBER: HTS055RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

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.

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 optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at CXCR5.

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

APPLICATION DATA

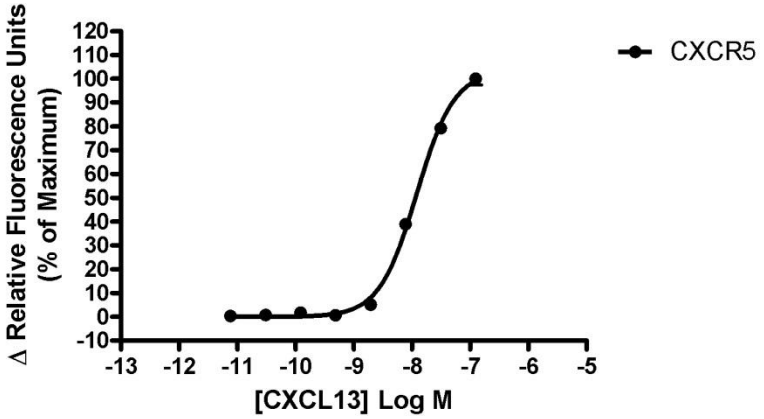

Figure 1. Representative data for activation of CXCR5 receptor. Calcium flux in CXCR5–expressing Chem-10 cell line induced by CXCL13. CXCR5–expressing Chem-10 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,700 RLU (Relative Light Units).

Table 1. Comparison of EC_{50} values of CXCL13-expressing Chem-10 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
CXCL13	Calcium Flux	12	Eurofins Internal Data
CXCL13	Calcium Flux	100	Gunn et al., 1998

ASSAY SETUP

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% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
CXCL13/BLC/BCA-1 ligand	R&D Systems: 201-CX
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

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	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-10, an adherent rat hematopoietic cell line expressing endogenous G·15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

CXCR5 cDNA (Accession Number: NM_001716; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                  ATG AAC TAC CCG CTA ACG CTG GAA
ATG GAC CTC GAG AAC CTG GAG GAC CTG TTC TGG GAA CTG GAC AGA TTG GAC AAC TAT AAC GAC
ACC TCC CTG GTG GAA AAT CAT CTC TGC CCT GCC ACA GAG GGG CCC CTC ATG GCC TCC TTC AAG
GCC GTG TTC GTG CCC GTG GCC TAC AGC CTC ATC TTC CTC CTG GGC GTG ATC GGC AAC GTC CTG
GTG CTG GTG ATC CTG GAG CGG CAC CGG CAG ACA CGC AGT TCC ACG GAG ACC ATC CTG TTC CAC
CTG GCC GTG GCC GAC CTC CTG CTG GTC TTC ATC TTG CCC TTT GCC GTG GCC GAG GGC TCT GTG
GGC TGG GTC CTG GGG ACC TTC CTC TGC AAA ACT GTG ATT GCC CTG CAC AAA GTC AAC TTC TAC
TGC AGC AGC CTG CTC CTG GCC TGC ATC GCC GTG GAC CGC TAC CTG GCC ATT GTC CAC GCC GTC
CAT ACC TAC CGC CAC CGC CGC CTC CTC TCC ATC CAC ATC ACC TGT GGG ACC ATC TGG CTG GTG
GGC TTC CTC CTT GCC TTG CCA GAG ATT CTC TTC GCC AAA GTC AGC CAA GGC CAT CAC AAC AAC
TCC CTG CCA CGT TGC ACC TTC TCC CAA GAG AAC CAA GCA GAA ACG CAT GCC TGG TTC ACC TCC
CGA TTC CTC TAC CAT GTG GCG GGA TTC CTG CTG CCC ATG CTG GTG ATG GGC TGG TGC TAC GTG
GGG GTA GTG CAC AGG TTG CGC CAG GCC CAG CGG CGC CCT CAG CGG CAG AAG GCA GTC AGG GTG
GCC ATC CTG GTG ACA AGC ATC TTC TTC CTC TGC TGG TCA CCC TAC CAC ATC GTC ATC TTC CTG
GAC ACC CTG GCG AGG CTG AAG GCC GTG GAC AAT ACC TGC AAG CTG AAT GGC TCT CTC CCC GTG
GCC ATC ACC ATG TGT GAG TTC CTG GGC CTG GCC CAC TGC TGC CTC AAC CCC ATG CTC TAC ACT
TTC GCC GGC GTG AAG TTC CGC AGT GAC CTG TCG CGG CTC CTG ACC AAG CTG GGC TGT ACC GGC
CCT GCC TCC CTG TGC CAG CTC TTC CCT AGC TGG CGC AGG AGC AGT CTC TCT GAG TCA GAG AAT
GCC ACC TCT CTC ACC ACG TTC TGA
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS055M	ChemiScreen™ CXCR5 Chemokine receptor membrane prep

* Note: Chem-10 cells are derived from Chem-1 cells

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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