eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ CXCR6 Chemokine Receptor Frozen Cells

CATALOG NUMBER: HTS054RTA

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.

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). Millipore's 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 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 CXCR6.

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

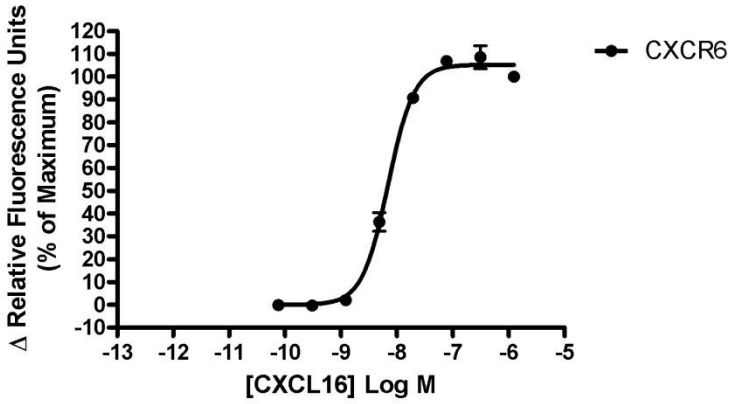

Figure 1. Representative data for activation of CXCR6 receptor. Calcium flux in CXCR6–expressing Chem-5 cell line induced by CXCL16. CXCR6–expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,600 RLU (Relative Light Units).

Table 1. Comparison of EC_{50} values of CXCR6-expressing Chem-5 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
CXCL16	Fluorescence	7	Eurofins Internal Data
CXCL16	Fluorescence	25	Wilbanks *et al*., 2001

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
CXCL16 ligand	Peprotech: 300-55
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-5, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

EXONGENOUS GENE EXPRESSION

CXCR6 cDNA (Accession Number: NM_006564; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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   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-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS054M	ChemiScreen™ CXCR6 Chemokine receptor membrane prep

* Note: Chem-5 cells are derived from Chem-1 cells

REFERENCES

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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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