

PRODUCT DATASHEET

Ready-to-Assay™ CCR5 Chemokine Family Receptor Frozen Cells

CATALOG NUMBER: HTS010RTA

Lot: 13122013

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 N₂. Media Component at 4°C (-20°C 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.

CCR5 is the receptor for CC chemokines MIP-1 α , MIP-1 β , and RANTES (Raport et al., 1996), and is preferentially expressed on Th1 lymphocytes (Loetscher et al., 1998). CCR5 is a coreceptor for macrophage-tropic HIV, and its ligands potently inhibit HIV replication in human leukocytes (Cocchi et al., 1995). In addition, HIV-infected patients with the nonfunctional CCR5 Δ 32 allele exhibit delayed onset of AIDS symptoms (Samson et al., 1996), and pharmacological antagonism of CCR5 inhibits HIV-1 infection (Strizki et al., 2001). Preclinical testing of small molecule antagonists of CCR5 has been hampered by low affinity of the compounds to rodent and dog CCR5, but two such compounds, maraviroc and AD101, have been shown to have potent antagonist activity at rhesus macaque CCR5, which differs from human CCR5 by 8 amino acids (Napier et al., 2005; Billick et al., 2004). One antagonist of human CCR5, SCH-C, does not block HIV entry through rhesus macaque CCR5, and one amino acid difference is responsible for the functional difference (Billick et al., 2004). The cloned rhesus macaque CCR5-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant CCR5 expression on the cell surface and contains high levels of the promiscuous G protein G α 15 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 CCR5.

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 genéticamente 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.

APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

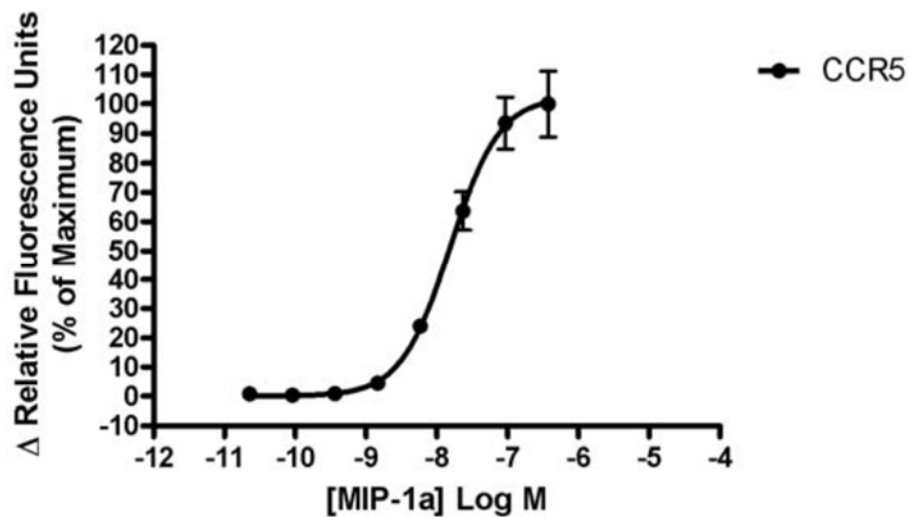


Figure 1. Representative data for activation of CCR5 receptor. Calcium flux in CCR5-expressing Chem-1 cell line induced by MIP-1 α . CCR5-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s) was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,200 RLU (Relative Light Units).

Table 1. EC50 values of CCR5-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
MIP-1 α	Calcium Flux	15	Eurofins Internal Data

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 37°C 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 μ L/well for 96-well plate, 25 μ L/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 37°C 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) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ 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 Ca²⁺ 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
Probenecid	Sigma: P8761
Quest Fluo-8 TM , AM	AAT Bioquest: 21080
MIP-1α ligand	Peptidech: 300-08-20
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-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein

EXONGENOUS GENE EXPRESSION

rhesus macaque CCR5 cDNA (Accession Number: NM_001042773.2; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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1 - ATGGAATATCAAGTGTCAAGTCCAACTATGACATCGATTATTATACATCGGAACCCCTGCCAAAAAATCAATGTGAAACAAATCGCAGCC - 90
1 - M D Y Q V S S P T Y D I D Y Y T S E P C Q K I N V K Q I A A - 30

1 - CGCCTCCTGCCTCCGCTCTACTCACTGGTGTTCATCTTTGGTTTGTGGGCAACATACTGGTCTCCTCATCTGATAAATGCAAAAGG - 180
1 - R L L P P L Y S L V F I F G F V G N I L V V L I L I N C K R - 60

1 - CTGAAAAGCATGACTGACATCTACCTGCCTCAACCTGGCCATCTCTGACCTGCTTTTCCTTCTTACTGTCCCTTCTGGGCTCACTATGCT - 270
1 - L K S M T D I Y L L N L A I S D L L F L L T V P F W A H Y A - 90

1 - GCTGCCAGCATGGGACTTTGGAAATACAATGTGTCAACTCTTGACAGGGCTCTATTTTATAGGCTTCTTCTGGAATCTTCTTCATCATC - 360
1 - A A Q W D F G N T M C Q L L T G L Y F I G F F S G I F F I I - 120

1 - CTCCTGACAATCGATAGGTACCTGGCTATCGTCCATGCTGTGTTGCTTTAAAGCCAGGACAGTCACCTTTGGGGTGGTACAAAGTGTG - 450
1 - L L T I D R Y L A I V H A V F A L K A R T V T F G V V T S V - 150

1 - ATCACTGGGTGGTGGCTGTGTTTGCCCTCTCTCCAGGAATCATCTTTACCAGATCTCAGAGAGAAGGTCTTCATTACACCTGCAGCTCT - 540
1 - I T W V V A V F A S L P G I I F T R S Q R E G L H Y T C S S - 180

1 - CATTTTCCATACAGTCAATCTGGAAGAATTTTCAGACATTAAGATGGTTCATCTGGGGCTGGTCCCGCTGCTTGTGCATG - 630
1 - H F P Y S Q Y Q F W K N F Q T L K M V I L G L V L P L L V M - 210

1 - GTCATCTGCTACTCGGGAACTCTGAAAACCTCTGCTTCGGTGTGCGAAACGAGAGAAGAGGGCACAGGGCTGTGAGGCTTATCTTCACCATC - 720
1 - V I C Y S G I L K T L L R C R N E K K R H R A V R L I F T I - 240

1 - ATGATTGTTTATTTTCTCTCTGGGCTCCCTACAACATGTCTCTCTCTGAAACACCTTCCAGGAATCTTTGGCCCTGAATAATTGCAGT - 810
1 - M I V Y F L F W A P Y N I V L L L N T F Q E F F G L N N C S - 270

1 - AGCTCTAACAGGTTGGACCAAGCCATGACAGGTGACAGAGACTCTGGGATGACACACTGTGTCATCAACCCCATCATCTATGCCTTCGTC - 900
1 - S S N R L D Q A M Q V T E T L G M T H C C I N P I I Y A F V - 300

1 - GGGGAGAAGTTCAGAACTACCTCTTAGTCTTCTTCCAAAAGCACATTGCCAAAACGCTTCTGCAAAATGCTGTTCCATTTTCCAGCAAGAG - 990
1 - G E K F R N Y L L V F F Q K H I A K R F C K C C S I F Q Q E - 330

1 - GCTCCCGAGCGAGCAAGTTCAGTTTACACCCGATCCACTGGGGAGCAGGAATATCTGTGGGCTTG TGA
1 - A P E R A S S V Y T R S T G E Q E I S V G L Stp
  
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS010M	ChemiScreen™ CCR5 chemokine family receptor membrane prep

REFERENCES

1. Billick E et al. (2004) The differential sensitivity of human and rhesus macaque CCR5 to small-molecule inhibitors of human immunodeficiency virus type 1 entry is explained by a single amino acid difference and suggests a mechanism of action for these inhibitors. *J. Virol.* 78: 4134-4144.
2. Cocchi F., et al. (1995) Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 270: 1811-1815.
3. Loetscher P, et al. (1998) CCR5 is characteristic of Th1 lymphocytes. *Nature* 391: 344-5.
4. Napier C et al. (2005) Molecular cloning and radioligand binding characterization of the chemokine receptor CCR5 from rhesus macaque and human. *Biochem. Pharmacol.* 71: 163-172.

5. Raport CJ et al. (1996) Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. J Biol Chem 271: 17161-6.
6. Samson M et al. (1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 382: 722-5.
7. Strizki JM et al. (2001) SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. Proc. Natl. Acad. Sci. USA 98: 12718-23.

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