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ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
RHESUS MACAQUE RECOMBINANT CCR5 CHEMOKINE RECEPTOR

CATALOG NUMBER: HTS010C

QUANTITY: 2 vials, 1 mL per vial

LOT NUMBER:

CONCENTRATION: 2 x 10^6 cells/mL

BACKGROUND:

CCR5 is the receptor for CC chemokines MIP-1α, MIP-1β, 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 CCR5Δ32 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). Millipore's 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 Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between CCR5 and its ligands.

APPLICATIONS:

Calcium flux assay, ligand binding assays

Figure 1. Calcium flux in CCR5–expressing Chem-1 cell line induced by MIP-1α and RANTES. CCR5–expressing Chem-1 cells and Wild-Type Chem-1 cells (Chemicon catalog # HTSCHEM-1) were loaded with DiscoverX NW and calcium flux in response to MIP-1α and RANTES (10^{-6} to $10^{-11.5}$ M) was determined in duplicate on a Molecular Devices FLIPR^{TETRA}™.

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SPECIFICATIONS: EC50 for calcium mobilization by MIP-1α: ~ 5.1 nM
EC50 for calcium mobilization by RANTES: ~ 2.0nM

HOST CELLS: Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

TRANSFECTION: Full-length rhesus CCR5 cDNA encoding CCR5 (Accession Number: NM_001042773.2)

PRESENTATION:

Cells are frozen at 2 x 10^6 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
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. Transfer contents of the vial to a T75 flask containing growth media. Place the flask in a humidified incubator at 37ºC with 5% CO_2.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca^{++} and Mg^{++} (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37ºC with 5% CO_2 until cells begin to round up and detach (5-10 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-1 Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10^6 cells/mL in Chem-1 Freezing Media (cell densities of 2-10 x 10^6 are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at –70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays.

MEDIA:

Chem-1 Growth Media*:*
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020 or equivalent)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)

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10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
G-418 (250ug/mL)

Chem-1 Plating Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
10% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep

Chem-1 Freezing Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine
20% heat-inactivated FBS
1x NEAA
10mM HEPES
1x Pen-Strep
10% DMSO (cell culture grade)

REFERENCES:

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.

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Loetscher P, *et al.* (1998) CCR5 is characteristic of Th1 lymphocytes. *Nature* 391: 344-5.

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.

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.

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.

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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Product No. HTS010C

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