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# ChemiSCREEN™ Human Recombinant XCR1 Chemokine Receptor Calcium-Optimized Stable Cell Line

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| **CATALOG NUMBER:** | HTS053C | **QUANTITY:** | 2 vials, 1 mL per vial |
| **LOT NUMBER:** | R0709E0016 | **CONCENTRATION:** | 2 x $10^6$ cells/mL |

**BACKGROUND:**

The migration of leukocytes from the bloodstream to sites of inflammation is a dynamic factor involving adhesion molecules and chemotactic factors. Chemokines play a role in the trafficking of leukocytes by inducing cellular motility and activating adhesion molecules within the immune system. Lymphotactin (also known as XCL1 and SCM-1) is a unique chemokine that retains only two of the four cysteine residues found in the CC, CXC and CX3C families of chemokines. A Gi-coupled receptor, XCR1, binds to lymphotactin and mediates its chemotactic effects (Yoshida *et al*., 1998). Chemokines promote accumulation of activated mononuclear cells (MNCs) in inflamed joints in rheumatoid arthritis (RA) and lymphotacin is highly expressed in synovial fluid of RA patients. In situ hybridization studies indicate that XCR1 expression was detected in both the infiltrated MNCs and the synoviocytes from synovial specimens taken from RA patients (Wang *et al.*, 2004). Millipore's cloned human XCR1-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant XCR1 expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between XCR1 and its ligands.

**APPLICATIONS:**

Calcium flux assay, ligand binding assays

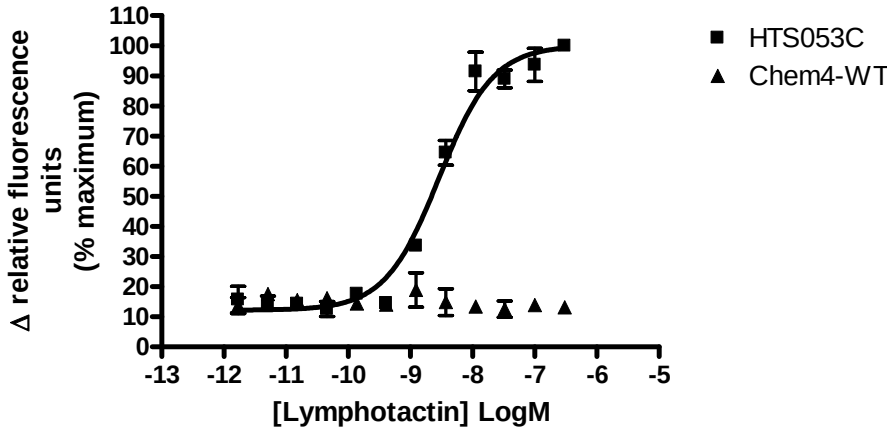


**Figure 1.** Calcium flux in XCR1–expressing Chem-4 cell line induced by Lymphotactin. XCR1 expressing Chem-4 cells and Wild-Type Chem-4 cells were loaded with Fluo-4 NW and calcium flux in response to Lymphotactin ($10^{-6.5}$ to $10^{-11.7}$M) was determined in triplicate on a Molecular Devices FLIPR TETRA.

SPECIFICATIONS: EC50 for calcium mobilization by Lymphotactin: ~3 nM

HOST CELLS: Chem-4, an adherent cell line expressing the promiscuous G-protein.

TRANSFECTION: Full-length human XCR1 cDNA encoding XCR1 (Accession Number: NM_005283)

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**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-4 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-4 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. Cells should be resuspended in Chem-4 Plating Media for plating for calcium assay.

**MEDIA:**

Chem-4 Growth Media:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
1x Pen-Strep (from 100x stock, Millipore TMS-AB2-C)
250μg/mL Genetecin/G-418
250μg/mL Hygromycin

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

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Chem-4 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:**

Wang CR *et al.* (2004) Up-regulation of XCR1 expression in rheumatoid joints. Rheumatology: 43: 569-73.

Yoshida T *et al.* (1998) Identification of single C motif-1/lymphotactin receptor XCR1. *J. Biol. Chem.* 273: 16551-16554.

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