Human XCR1/Galpha15 Stable Cell Line-Chem-4

Cat. No. CSC-RG0645 **Lot. No.** (See product label)

SPECIFICATION

Species	Human
Source	Chem-4
Cell Line Description	This 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.
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. 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.
Growth Properties	Adherent

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Host Cell	Chem-4
Cell Line Validation	1. Gene expression: qPCR experiments determined specific silencing of XCR1. 2. Protein expression: XCR1 in this cell line has been validated by immunocytochemical staining.3. EC50 for calcium mobilization by Lymphotactin: ~3 nM.
Application	Calcium flux assay, ligand binding assays
Sub-type	Chemokine
Propagation	Complete growth medium: DMEM with 4.5 g/L glucose + 4mM glutamine + 10% heat-inactivated FBS + 1x Nonessential amino acids + 10mM HEPES + 1x Pen-Strep + 250µg/mL Genetecin/G-418 + 250µg/mL Hygromycin Plating medium: DMEM with 4.5 g/L glucose + 4mM glutamine + 10% heat-inactivated FBS + 1x NEAA + 10mM HEPES + 1x Pen-Strep Atmosphere: air, 95%; carbon dioxide (CO2), 5%Temperature: 37 °C
Starting Cells From Frozen Cell Stock	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 37°C 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 37°C with 5% CO2. 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.
Subculturing	1. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca2+ and Mg2+ (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO2 until cells begin to round up and detach (5-10 minutes). Gently rap the

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	side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Growth media per 1 mL trypsin. 2. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
Mycoplasma	Mycoplasma Status: Negative (MycoAlert Kit)
Freeze Medium	Freezing medium: DMEM with 4.5 g/L glucose + 4mM glutamine + 20% heat-inactivated FBS + 1x NEAA + 10mM HEPES + 1x Pen-Strep + 10% DMSO
Storage	Liquid nitrogen
Preservation	1. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Subculture-Step 1). Centrifuge cells at 200 x g for 5 min. Resuspend cells at 5 x 10^6 cells/mL in 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. 2. 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 Plating Media for plating for calcium assay.
Safety Considerations	The following safety precautions should be observed.1. Use pipette aids to prevent ingestion and keep aerosols down to a minimum.2. No eating, drinking or smoking while handling the stable line.3. Wash hands after handling the stable line and before leaving the lab.4. Decontaminate work surface with disinfectant or 70% ethanol before and after working with stable cells.5. All waste should be considered hazardous.6. Dispose of all liquid waste after each experiment and treat with bleach.
Ship	Dry ice

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GENE INFORMATION

Gene Name	XCR1 chemokine (C motif) receptor 1 [Homo sapiens]
Official Symbol	XCR1
Synonyms	XCR1; chemokine (C motif) receptor 1; CCXCR1, chemokine (C motif) XC receptor 1 , GPR5; chemokine XC receptor 1; lymphotactin receptor; XC chemokine receptor 1; G protein-coupled receptor 5; G-protein coupled receptor 5; chemokine (C motif) XC receptor 1; GPR5; CCXCR1;
Gene ID	2829
mRNA Refseq	NM_001024644
Protein Refseq	NP_001019815
MIM	600552
UniProt ID	P46094
Chromosome Location	3p21.3-p21.1
Pathway	Chemokine receptors bind chemokines, organism-specific biosystem; Chemokine signaling pathway, organism-specific biosystem; Chemokine signaling pathway, conserved biosystem; Class A/1 (Rhodopsin-like receptors), organism-specific biosystem; Cytokine-cytokine receptor interaction, organism-specific biosystem; Cytokine-cytokine receptor interaction, conserved biosystem; G alpha (q) signalling events, organism-specific biosystem;

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Function	G-protein coupled receptor activity; chemokine receptor activity; receptor activity; signal transducer activity;

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