

PRODUCT DATASHEET

ChemiScreen™ CX₃CR1 Chemokine Receptor Stable Cell Line

CATALOG NUMBER: HTS015C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N₂.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-4 host, which supports high levels of functional receptor expression on the cell surface. Chem-4 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

CX₃CR1 is a GPCR expressed on natural killer cells, cytotoxic T lymphocytes, and macrophages. The sole ligand for CX₃CR1, fractalkine, is an unusual chemokine that is expressed as a transmembrane molecule with a CX3C domain and a mucin domain (Imai *et al.*, 1997). Fractalkine is highly expressed on endothelial cells activated by TNFα and other proinflammatory cytokines, and fractalkine/CX₃CR1 interactions mediate recruitment of macrophages into the atherosclerotic plaque (Lesnick *et al.*, 2003; McDermott *et al.*, 2003). In addition, fractalkine and CX₃CR1 have been implicated in the pathogenesis of glomerulonephritis, HIV infection and rheumatoid arthritis (Ito *et al.*, 2002; Faure *et al.*, 2003; Nanki *et al.*, 2002). The cloned human CX₃CR1 –expressing cell line is made in the Chem-4 host, which supports high levels of recombinant CX₃CR1 expression on the cell surface and contains high levels of promiscuous G proteins to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between CX₃CR1 and its ligands.

USE RESTRICTIONS

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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.

APPLICATIONS

Calcium Flux Fluorescence Assay

APPLICATION DATA

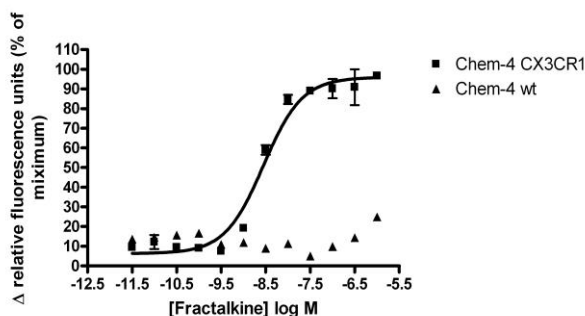


Figure 1. Representative data for activation of CX₃CR1 receptor stably expressed in Chem-4 cells induced by Fractalkine using a fluorescent calcium flux assay. CX₃CR1-expressing Chem-4 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-4) were tested to determine the specificity of the resulting signal.

Table 1. EC₅₀ value of CX₃CR1-expressing Chem-4 cells.

| LIGAND | ASSAY | POTENCY EC ₅₀ (nM) | REFERENCE |
|-------------|-----------------------------|-------------------------------|------------------------|
| Fractalkine | Calcium Flux – Fluorescence | 72.8 | Eurofins Internal Data |

* The cell line was tested and found to have equivalent EC₅₀ and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

| Description | Component | Concentration | Supplier and Product Number |
|-------------------------|-----------------------------------|---------------|-----------------------------|
| Basal Medium | DMEM high glucose Medium (4.5g/L) | - | Hyclone: SH30022 |
| | Fetal Bovine Serum (FBS) | 10% | Hyclone: SH30070.03 |
| | Non-Essential Amino Acids (NEAA) | 1X | Hyclone: SH30238.01 |
| | HEPES | 1X | EMD Millipore: TMS-003-C |
| Selection Medium | Basal Medium (see above) | - | |
| | Geneticin (G418) | 250 µg/ml | Invivogen: ant-gn-5 |
| Dissociation | Hygromycin | 500 µg/ml | Invivogen: ant-hg-5 |
| | Sterile PBS | - | Hyclone: SH30028.03 |
| | 0.25% Trypsin-EDTA | - | Hyclone: SH30042.01 |
| CryoMedium | Basal Medium (see above) | 40% | |
| | Fetal Bovine Serum (FBS) | 50% | Hyclone: SH30070.03 |
| | Dimethyl Sulfoxide (DMSO) | 10% | Sigma: D2650 |

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37°C Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37°C water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37°C, 5% CO₂.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%. At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37° C incubator for additional 2 min.* Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

| Flask Size (cm ²) | Volume (mL) | Total Cell Number (x10 ⁶) | Growth Period (hrs) |
|-------------------------------|-------------|---------------------------------------|---------------------|
| T75 | 15 | 5.0 | 24 |
| T75 | 15 | 2.0 | 48 |
| T75 | 15 | 0.45 | 72 |

ASSAY SETUP

Fluorescence

Table 4. 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 | 95 µl (50 µl for 384-well) |
| Dispense Speed | 50 µl/sec |
| Expel Volume | 0 µl |
| Analysis | Subtract Bias Sample 1 |

Table 5. Assay Materials (Not provided)

| Description | Supplier and Product Number |
|--------------------------------------------------|-----------------------------|
| HBSS | Invitrogen: 14025 |
| HEPES 1M Stock | EMD Millipore: TMS-003-C |
| Probenicid | Sigma: P8761 |
| Quest Fluo-8™, AM | AAT Bioquest: 21080 |
| Fractalkine ligand | Peprotech: 300-31 |
| Non-Binding 96/384 well Plates (for ligand prep) | Corning: 3605/ 3574 |
| Black (clear Bottom) cell assay plates | Corning: 3904/ 3712 |
| Coelenterazine-h (250µg). Prepare to 10mM | Promega: S2011 |

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5×10^5 cells/ml (i.e, if collected 5×10^6 TC, $\frac{5 \times 10^6}{5 \times 10^5/ml} = 10$ mL volume)
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37°C 5% CO₂ incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-4, an adherent cell line expressing the promiscuous G-protein, G α 15.

EXOGENOUS GENE EXPRESSION

Human CX₃CR1 cDNA (Accession Number: U28934) and promiscuous G protein are expressed in a bicistronic vector

RELATED PRODUCTS

| Product Number | Description |
|----------------|-------------------------------------------------------------------|
| HTSCHEM-1 | ChemiScreen™ Chem-1 Parental Cell Line (control cells) |
| HTS015M | ChemiScreen™ CX ₃ CR1 Chemokine Receptor Membrane Prep |

REFERENCES

1. Faure S. *et al.* (2003) Deleterious genetic influence of CX3CR1 genotypes on HIV-1 disease progression. *J Acquir. Immune Defic. Syndr.* 32: 335-7 .
2. Imai T. *et al.* (1997) Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion. *Cell* 91: 521-30.
3. Ito Y. *et al.* (2002) Fractalkine expression and the recruitment of CX3CR1+ cells in the prolonged mesangial proliferative glomerulonephritis. *Kidney Int.* 61: 2044-57.
4. Lesnick P. *et al.* (2003) Decreased atherosclerosis in CX3CR1-/- mice reveals a role for fractalkine in atherogenesis. *J. Clin. Invest.* 111: 333-40.
5. McDermott D.H. *et al.* (2003) Chemokine receptor mutant CX3CR1-M280 has impaired adhesive function and correlates with protection from cardiovascular disease in humans. *J. Clin. Invest.* 111: 1241-50.
6. Nanki T. *et al.* (2002) Migration of CX3CR1-positive T cells producing type 1 cytokines and cytotoxic molecules into the synovium of patients with rheumatoid arthritis. *Arthritis Rheum.* 46: 2878-83.

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