

## READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS HUMAN RECOMBINANT CCR1 CHEMOKINE RECEPTOR

**CATALOG NUMBER:** HTS005F      **QUANTITY:** 1 vial, 1 mL  
**LOT NUMBER:**      **CONCENTRATION:** 1 x 10<sup>7</sup> viable cells/mL

**BACKGROUND:** Millipore's Ready-To-Assay Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay cells is identical to that of the originating GPCR cell line.

CCR1 is a GPCR that binds to a variety of CC ligands, including MIP-1α, RANTES, MCP-3, HCC-1, HCC-2, HCC-4, and MPIF-1 (Olson and Ley, 2002). Lymphocytes, macrophages, dendritic cells, and GM-CSF-activated neutrophils express CCR1 (Kaufmann *et al.*, 2001; Cheng *et al.*, 2001). Two selective, non-peptide small molecule antagonists of CCR1, BX-471 and CP-481,715, have been synthesized (Gladue *et al.*, 2003; Liang *et al.*, 2000). Pharmacological and genetic targeting of CCR1, either alone or in combination with cyclosporin A, reduces cardiac and renal allograft rejection (Gao *et al.*, 2000; Horuk *et al.*, 2001a; Horuk *et al.*, 2001b), allergic encephalomyelitis (Liang *et al.*, 2000), and renal fibrosis (Anders *et al.*, 2002) in experimental models. Millipore's cloned human CCR1-expressing cells are made in the Chem-1 host, an adherent cell line. The untreated CCR1-Chem-1 cell line and the Ready-To-Assay CCR1 cells have equivalent EC50s for MIP-1α.

**APPLICATIONS:** Calcium flux assay

**SPECIFICATIONS:**

**PRESENTATION:**

	EC50 for MIP-1α	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	4.9 nM	1940	0.69
Continuous Passage Cells	7.8 nM	1784	0.52

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

**TRANSFECTION:** Full-length human CCR1 cDNA (Accession Number: L09230)

**PLATING 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)
- 100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

Cells are frozen at 1 x 10<sup>7</sup> cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and

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streptomycin/10% DMSO.

**STORAGE:**

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

**ASSAY PROTOCOL:**

- 1) 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.
- 2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
- 3) Resuspend cells to  $0.5 \times 10^6$  cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200  $\mu$ L per well to obtain a density of approximately  $1 \times 10^5$  cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO<sub>2</sub>.
- 5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

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