

**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT CXCR2 CHEMOKINE RECEPTOR**

CATALOG NUMBER:	HTS002F	QUANTITY:	1 vial, 1 mL
LOT NUMBER:		CONCENTRATION:	1 x 10 ⁷ 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.

CXCR2 is a 7-TM G-protein coupled receptor that binds to the chemokines GROα, GROβ, GROγ, IL-8, ENA-78, NAP-2 and GCP-2 (Olson and Ley, 2002). Neutrophils, mast cells and microvascular endothelial cells express CXCR2, and interactions of CXCR2 with its ligands promotes chemotaxis of these cell types (Heidemann *et al.*, 2003; Nilsson *et al.*, 1999; White *et al.*, 1998). Studies with mice lacking CXCR2 indicate that CXCR2 promotes growth of primary tumors and secondary metastases (Keane *et al.*, 2004), and plays an essential role in hyperoxia-induced lung injury (Sue *et al.*, 2004). In addition, cytomegalovirus encodes a CXCR2-binding chemokine, vCXC-1, that promotes neutrophil migration to infected cells (Penfold *et al.*, 1999). Millipore's cloned CXCR2-expressing cell line is made in the Chem-1 host, an adherent cell line that supports high levels of recombinant CXCR2 expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated CXCR2-Chem-1 cell line and the Ready-To-Assay™ CXCR2 cells have equivalent EC50s for IL-8.

APPLICATIONS: Calcium flux assay

SPECIFICATIONS:

	EC50 for IL-8 (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	2.22	3468	0.55
Continuous Passage Cells	0.73	5013	0.80

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

- PRESENTATION:** TRANSFECTION: Full-length human CXCR2 cDNA (Accession Number: M73969)
- 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 1×10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and 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 0.5×10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 μ L per well to obtain a density of approximately 1×10^5 cells/well.
 - 4) Place the assay plate in a humidified 37°C incubator with 5% CO₂.
 - 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.
- REFERENCES:**
- Heidemann J, *et al.* (2003) Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. *J. Biol. Chem.* 278: 8508-15
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- Nilsson G, *et al.* (1999) Mast cell migratory response to interleukin-8 is mediated through interaction with chemokine receptor CXCR2/Interleukin-8RB. *Blood* 93: 2791-7
- Olson TS and Ley K (2002) Chemokines and chemokine receptors in leukocyte trafficking. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 283: R7-28
- Penfold ME, *et al.* (1999) Cytomegalovirus encodes a potent alpha chemokine. *Proc Natl Acad Sci USA* 96: 9839-44
- Sue RD, *et al.* (2004) CXCR2 is critical to hyperoxia-induced lung injury. *J. Immunol.* 172: 3860-8
- White JR, *et al.* (1998) Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. *J. Biol. Chem.* 273: 10095-8

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HUMAN RECOMBINANT CXCR2 CHEMOKINE RECEPTOR
Product No. HTS002F**

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