MILLIPORE

CHEMICON
now part of Millipore

READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT CXCR3 CHEMOKINE RECEPTOR

CATALOG NUMBER:	HTS003F	**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.

CXCR3 is a 7-TM GPCR that is selective for the CXC chemokines IP10, I-TAC and MIG (Loetscher *et al.*, 1996). Binding of IP10 and MIG to CXCR3 induces Ca^{2+} mobilization, chemotaxis and inflammatory responses of T lymphocytes, and also act as potent inhibitors of angiogenesis. CXCR3 is highly expressed in IL-2-activated T lymphocytes in vitro (Loetscher *et al.,* 1996), and in T lymphocytes present in inflamed tissues in rheumatoid arthritis and multiple sclerosis (Balashoy *et al.*, 1999; Qin *et al.*, 1998). In vivo, neutralization of CXCR3 inhibits experimentally induced type I diabetes (Frigerio *et al.*, 2002), peritonitis (Xie *et al.*, 2003), and post-lung transplantation bronchiolitis obliterans syndrome (Belperio *et al.*, 2002). Millipore's cloned human CXCR3-expressing cells are made in the Chem-1 host, an adherent cell line. The untreated CXCR3-Chem-1 cell line and the Ready-To-Assay CXCR3 cells have equivalent EC50s for IP10.

APPLICATIONS:

Calcium flux assay

SPECIFICATIONS:

	EC50 for IP10 nM	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	4.98 nM	2604	0.71
Continuous Passage Cells	2.47 nM	3142	0.55

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

TRANSFECTION: Full-length human cDNA encoding CXCR3 (Accession Number: X95876)

PLATING MEDIA:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com

MILLIPORE

CHEMICON

now part of Millipore

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)

PRESENTATION:

Cells are frozen at 1 x 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 37ºC 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 x 10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 µL per well to obtain a density of approximately 1 x 10^5 cells/well.
4) Place the assay plate in a humidified 37°C incubator with 5% CO_2.
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:

Loetscher M, *et al*. (1996) Chemokine receptor specific for IP10 and mig: structure, function, and expression in activated T-lymphocytes. *J Exp Med.* 184: 963-9.

Balashov, KE, *et al.* (1999) CCR5 (+) and CXCR3 (+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions.*Proc. Natl. Acad. Sci. USA* 96: 6873-8.

Qin, S, *et al.* (1998) The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions.*J. Clin. Invest.* 101: 746-54.

Frigerio, S, *et al.* (2002) Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulitis. *Nat. Med.* 8: 1414-20.

Xie, JH, *et al.* (2003) Antibody-mediated blockade of the CXCR3 chemokine receptor results in diminished recruitment of T helper 1 cells into sites of inflammation. *J. Leukoc. Biol.* 73: 771-7-80.

Belperio, JA, *et al.* (2002) Critical role for CXCR3 chemokine biology in the pathogenesis of bronchiolitis obliterans syndrome. *J. Immunol.* 169: 1037-1049.

For research use only; not for use as a diagnostic.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
Australia +61 3 9839 2000
www.millipore.com

MILLIPORE

CHEMICON

now part of Millipore

User Agreement (Label License) for
READY-TO-ASSAY CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT CXCR3 CHEMOKINE RECEPTOR
Product No. HTS003F

BY USING THE THIS PRODUCT LICENSED TO YOU ("LICENSEE") HEREUNDER, YOU ARE HEREBY REPRESENTING THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF OR YOUR COMPANY, AS APPLICABLE, AND ARE CONSENTING TO BE LEGALLY BOUND BY ALL OF THE TERMS OF THIS USER AGREEMENT ("AGREEMENT"). IF YOU DO NOT AGREE TO ALL THESE TERMS, DO NOT USE THE PRODUCT, AND IMMEDIATELY RETURN SUCH PRODUCTS TO THE APPLICABLE SELLER FOR A REFUND. This is a legal agreement between Licensee and Millipore governing use of the Ready-to-Assay Cells products and/or any accompanying operating/use protocols (the "Product(s)") provided to Licensee.

LICENSEE shall obtain no ownership interest in the Product or use/culture protocols accompanying the Product other than through the perpetual limited license granted herein. If the Product is licensed through an authorized Millipore distributor, Licensee shall be obligated to disclose its identity to Millipore to insure compliance with this User Agreement.

Limited License and Restrictions. Pursuant to the terms and conditions of this Agreement, Millipore conveys to Licensee the non-exclusive and non-transferable right to use the Licensed Product only for Research Purposes conducted by Licensee (whether Licensee is an academic user or a for-profit entity). "Research Purposes" means any biological research and development application or use, including without limitation, developing, demonstrating or validating biological assays, life sciences and/or pharmaceutical research. "Research Purposes" excludes applications outside biology (including but not limited to consumer electronics or materials sciences), and specifically excludes the following applications of whatever kind or nature: Clinical Diagnostics (any use of a product or service for clinical diagnosis where data from an individual's sample is given to such individual or used for the purpose of diagnosis or treatment of a medical condition in such individual, where that result may be used in the treatment of such individual), therapeutics, clinical imaging, environmental testing and cosmetics. Contents of the supplied vial(s) are limited to a single use and shall not be propagated by licensee. Licensee cannot sell or otherwise transfer (a) this Product or (b) materials made using this Product to a third party. Licensee may transfer information or materials made through use of this Product to a scientific collaborator, provided that such transfer is not for the commercial purposes, and that such collaborator agrees in writing: (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for Research Purposes and not for commercial purposes. Commercial purposes means any activity by a user of the Product for consideration that may include, but is not limited to: (1) operating a service business that uses the Products to develop information or data which is resold for research and development applications; (2) use of the Product in manufacturing; (3) use of the Product for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the Product, whether or not such Product is resold for use in research. Licensee expressly represents and warrants to Millipore that Licensee will properly test and use any Product purchased from Millipore or its affiliated companies in accordance with the practices of a reasonable person who is an expert in the field and in strict compliance with all applicable laws and regulations, now and hereinafter enacted. Licensee agrees to comply with instructions, if any, furnished by Millipore relating to the use of the Product and to not misuse the Product in any manner. Licensee shall not reverse engineer, disassemble or modify the Product or create any derivative works of the written materials accompanying the Product, including but not limited to any material data sheets or similar materials with respect to the Products' specifications. Licensee acknowledges that Millipore or its affiliated companies retains ownership of all patents, copyrights, trademarks, trade secrets and other proprietary rights relating to or residing in the Product or any portion thereof.

Licensee's Representations. Licensee agrees, and further represents and warrants: (i) that it shall use all Products solely in accordance with this Agreement, and that any such use of Products will not violate any applicable law, regulation, judicial order, or injunction; and (ii) that it is not prohibited from receiving the Products under U.S. export laws, that it is not a national of a country subject to U.S. trade sanctions, that it will not use the Products in a location that is the subject of U.S. trade sanctions that would cover the Products, and that, to its knowledge, it is not on the U.S. Department of Commerce's table of deny orders or is otherwise prohibited from obtaining goods of this sort from the United States.

No Warranties. TO THE MAXIMUM EXTENT PERMITTED BY APPLICABLE LAW, MILLIPORE AND ITS AFFILATED COMPANIES DO NOT WARRANT THAT THE USE OF THE PRODUCTS DELIVERED HEREUNDER WILL NOT INFRINGE THE CLAIMS OF ANY UNITED STATES OR OTHER PATENTS COVERING THE PRODUCT THEMSELVES OR THE USE THEREOF IN COMBINATION WITH OTHER PRODUCTS OR IN THE OPERATION OF ANY PROCESS. IN ADDITION, THE PRODUCTS ARE PROVIDED "AS IS," WITHOUT WARRANTY OF ANY KIND, AND MILLIPORE MAKES NO

WARRANTIES, WHETHER EXPRESS, IMPLIED, STATUTORY OR OTHERWISE, WITH RESPECT TO THE PRODUCTS OR THE USE THEREOF. MILLIPORE AND ITS AGENTS HEREBY SPECIFICALLY DISCLAIM THE IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, NON-INFRINGEMENT, ACCURACY, TITLE AND THE IMPLIED CONDITION OF SATISFACTORY QUALITY. LICENSEE ASSUMES ALL RESPONSIBILITIES FOR SELECTION OF THE PRODUCT TO ACHIEVE ITS INTENDED RESULTS, AND FOR THE USE OF THE PRODUCT.

Term and Termination. This Agreement commences upon Licensee's use of the Products, and shall remain in effect in perpetuity unless terminated sooner as set forth hereunder. Millipore may terminate this Agreement immediately if Licensee breaches any provision herein. Upon any such termination, all rights granted to Licensee hereunder will immediately terminate, and Licensee shall immediately cease using the Product and, at Millipore's option, return or destroy all Products (certifying such destruction to Millipore in writing).

Assignment. Licensee shall not sublicense, assign (by operation of law of otherwise) or otherwise transfer this Agreement or any of the rights or licenses granted under this Agreement without the prior written consent of Millipore. Any attempted assignment, sublicense or transfer by Licensee without such consent shall be null and void.

Miscellaneous. This Agreement constitutes the entire agreement between Millipore and Licensee, and no modification or amendment shall be effective unless signed in writing by authorized representatives of both parties. Millipore's failure to strictly enforce any term or condition of this order or to exercise any right, power, or privilege arising hereunder shall not constitute a waiver of Millipore's right to strictly enforce such terms or conditions or exercise such right, power, or privilege thereafter. All rights and remedies under this order are cumulative and are in addition to any other rights and remedies Millipore may have at law or in equity. Any waiver or default by Licensee hereunder shall be in writing and shall not operate as a waiver of any other default or of the same default thereafter. If any provision of this Agreement shall be held invalid, illegal or unenforceable, the validity, legality and enforceability of the remaining provisions, rights, powers, and privileges shall not be affected or impaired thereby. The paragraph headings herein are for convenience only and form no part of the terms and conditions and shall not affect the interpretation of the terms and conditions. This Agreement shall be binding upon, inure to the benefit of, and be enforceable by, the parties hereto, and their respective heirs, personal representatives, corporate representatives, agents, successors, and assigns. THIS AGREEMENT SHALL BE GOVERNED BY THE LAWS OF THE STATE OF CALIFORNIA, WITHOUT REFERENCE TO CONFLICT OF LAWS PRINCIPLES. ALL DISPUTES ARISING OUT OF OR RELATED TO THIS AGREEMENT WILL BE SUBJECT TO THE EXCLUSIVE JURISDICTION AND VENUE OF THE CALIFORNIA STATE COURTS OF SAN DIEGO COUNTY, CALIFORNIA (OR, IF THERE IS EXCLUSIVE FEDERAL JURISDICTION, A UNITED STATES SOUTHERN DISTRICT COURT OF CALIFORNIA), AND THE PARTIES CONSENT TO THE PERSONAL AND EXCLUSIVE JURISDICTION OF THESE COURTS.