

**ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT A₁ RECEPTOR**

CATALOG NUMBER:	HTS047C	QUANTITY:	2 vials, 1 mL per vial
LOT NUMBER:		CONCENTRATION:	2 x 10 ⁶ cells/mL

BACKGROUND: Extracellular adenosine mediates a multitude of biological effects, including wakefulness, antiarrhythmia, bronchoconstriction and response to ischemia and oxidative stress. A family of four GPCR adenosine receptors, A₁, A_{2A}, A_{2B} and A₃, is responsible for these effects. The A₁ receptor, which couples to G_{i/o}, is most highly expressed in brain, and mediates endogenous antinociception and neuronal response to hypoxia (Fredholm *et al.*, 2001). A₁ is also expressed in kidney, where it contributes to tubuloglomerular feedback. Chemicon's cloned human A₁-expressing cell line is made in the Chem-3 host, which supports high levels of recombinant A₁ expression on the cell surface and contains high levels of the promiscuous G protein G_α15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between A₁ and its ligands.

APPLICATIONS: Calcium flux assay, ligand binding assays

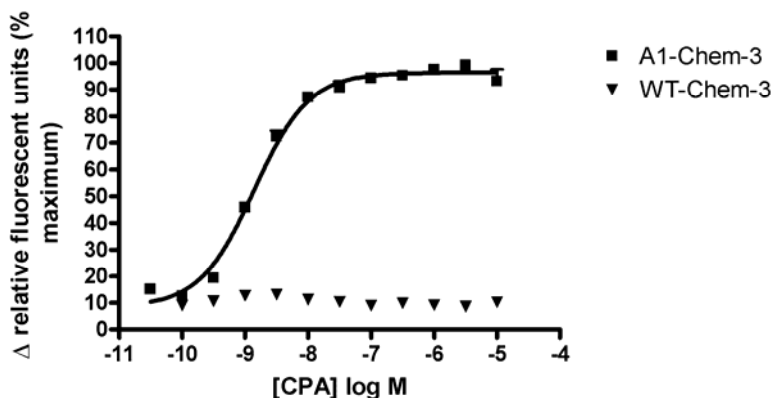


Figure 1. Calcium flux in A₁-expressing Chem-3 cell line induced by N⁶-cyclopentyladenosine (CPA). A₁-expressing Chem-3 cells and wild-type Chem-3 cells (WT-Chem-3) were loaded with Fluo-4 and calcium flux in response to CPA (10⁻⁵ to 10⁻¹⁰ M) was determined in triplicate on a Molecular Devices FLIPR^{TETRA}™.

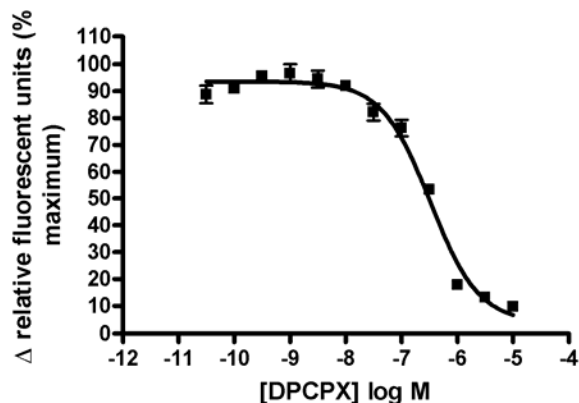


Figure 2. Assay for antagonist activity at A_1 by calcium flux assay. A_1 -expressing Chem-3 cells were loaded with Fluo-4 and washed. DPCPX was added to the cells at the final concentration indicated, and incubated for 10 min at 37°C. Calcium flux in response to CPA (3 nM) was determined in triplicate on a Molecular Devices FLIPR^{TETRA™}.

SPECIFICATIONS: EC50 for calcium mobilization by CPA: ~ 0.8 nM
 IC50 for DPCPX: ~250 nM
 Signal:noise ratio at CPA E_{max} : 747

HOST CELLS: Chem-3, a suspension cell line endogenously expressing the promiscuous G protein, $G_{\alpha 15}$, and lacking endogenous expression of adenosine receptors.

TRANSFECTION: Full-length human ADORA1 cDNA encoding A_1 (Accession Number: S45235)

GROWTH MEDIA: RPMI containing 4.5 g/L glucose/10% heat inactivated fetal bovine serum/0.8 mg/ml Geneticin (G418)/100 U/ml each penicillin and streptomycin, containing 1:100 dilution of Chem-3 Growth Supplement (Chemicon catalog # HTSCHEM-3S).

PRESENTATION:

Cells are frozen at 2×10^6 cells/mL in RPMI/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO. Cell line tests negative for mycoplasma.

STORAGE/HANDLING:

Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years. 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 sterile conical tube containing 10 mL growth media, and centrifuge at 200 x g. Remove supernatant and resuspend in fresh growth media. Transfer to a T75 flask containing 20 mL final volume growth media. Cells should be maintained between 0.1×10^6 and 1.0×10^6 cells/mL, and are typically passaged 1:10 every 3-4 days. Cells should be passaged at least once after thawing prior to use in calcium flux assays.

REFERENCES:

Fredholm, BB *et al.* (2001) International Union of Pharmacology. XXV. Nomenclature and classification of adenosine receptors. *Pharmacol. Rev.* 53: 527-552.

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

© 2002-2004 CHEMICON® International, Inc. - By CHEMICON® International, Inc. All rights reserved. No part of these works may be reproduced in any form without permissions in writing.

USA & Canada • Phone: +1(800) 437-7500 • Fax: +1 (951) 676-9209 • Europe +44 (0) 23 8026 2233
 Australia +61 3 9839 2000 • Germany +49-6192-207300 • ISO Registered worldwide
www.chemicon.com • custserv@chemicon.com • techserv@chemicon.com

**User Agreement (Label License) for
ChemiScreen™ CALCIUM-OPTIMIZED STABLE CELL LINE
HUMAN RECOMBINANT A₁ RECEPTOR**

Product No. HTS047C

BY USING 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 ChemiScreen™ Calcium-Optimized Stable GPCR cell line 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. 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 AFFILIATED 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 or 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.