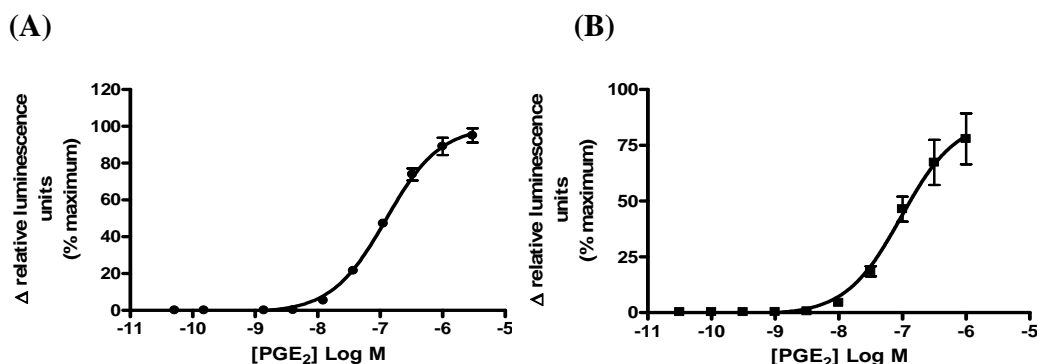


## ChemiScreen™ FLASH AEQUORIN CALCIUM-OPTIMIZED STABLE CELL LINE HUMAN RECOMBINANT EP<sub>2</sub> PROSTANOID RECEPTOR

<b>CATALOG NUMBER:</b>	HTS185AF	<b>QUANTITY:</b>	2 vials, 1 mL per vial
<b>LOT NUMBER:</b>	R0712E0023	<b>CONCENTRATION:</b>	2 x 10 <sup>6</sup> cells/mL

**BACKGROUND:** Prostanoids bind to a family of 8 GPCRs to exert their biological effects (Narumiya and FitzGerald, 2001). The prostanoid PGE<sub>2</sub> causes pain, vasodilation, immunosuppression of T cells, bone resorption and promotion of carcinogenesis. Four related GPCRs, EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub>, each bind to PGE<sub>2</sub>, but the different G protein coupling status of each receptor leads to distinct biological effects. EP<sub>2</sub> couples primarily to G<sub>s</sub> to increase intracellular cAMP levels. Mice deficient in EP<sub>2</sub> receptor showed impaired ovulation and fertilization, and salt-sensitive hypertension (Kennedy *et al.*, 1999). It has been shown that EP<sub>2</sub> receptors are also involved in cancer-associated immunodeficiency. Thus, genetic knockout of the EP<sub>2</sub> receptor reduced tumor growth and prolonged survival in mice that had undergone isograft injection of MC26 or Lewis lung carcinoma cells (Yang *et al.*, 2003). Millipore's cloned human EP<sub>2</sub>-expressing cell line is made in the Chem-9 host which stably expresses a mitochondrially targeted flash mutant form of aequorin. The purified version of this flash variant of aequorin has shown a higher luminescent signal intensity than purified wildtype aequorin. Thus, the cell line is an ideal tool for screening for agonists and antagonists at EP<sub>2</sub>.

**APPLICATIONS:** Luminescent and fluorescent calcium flux assays, ligand binding assays



**Figure 1.** Ligand-induced calcium flux in Flash Aequorin Chem-9 cell line stably transfected with EP<sub>2</sub>. Flash Aequorin Chem-9 stably co-expressing EP<sub>2</sub> were loaded with 5 μM coelenterazine for 3 h at room temperature. Luminescence in response to PGE<sub>2</sub> was determined (A) in quadruplicate in a 384 well plate on a FLIPR<sup>TETRA</sup> with aequorin option from Molecular Devices, now part of MDS Analytical Technologies. Data were collected for area under curve for 70 sec. (B) PGE<sub>2</sub>-induced luminescence was determined in duplicate in a 96 well plate with a PerkinElmer Wallac Victor2. Data were collected for area under curve for 20 sec.

**SPECIFICATIONS:** EC<sub>50</sub> for calcium mobilization by PGE<sub>2</sub>:  
~ 123.8 nM (FLIPR<sup>TETRA</sup>) and ~ 95.4 nM (Wallac Victor2)

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

HOST CELLS: Chem-9, an adherent cell line expressing the promiscuous G-protein.

TRANSFECTION: Full-length human PTGER2 cDNA encoding EP<sub>2</sub> (Accession Number: NM\_000956)

**PRESENTATION:** Cells are frozen at  $2 \times 10^6$  cells/mL in 90% fetal bovine serum/10% DMSO. Cell line tests negative for mycoplasma.

**STORAGE/HANDLING**  
:

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen. Maintain frozen in liquid nitrogen for up to 5 years.
2. 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 T75 flask containing growth media. Place the flask in a humidified incubator at 37°C with 5% CO<sub>2</sub>.
3. After 8-24 h, all live cells will be attached. Viability of the cells is expected to be 50-80%. At this time, replace media to remove residual DMSO, and return to incubator.
4. When cells are approximately 80% confluent, passage the cells as follows: Remove media and wash once with HBSS without Ca<sup>++</sup> and Mg<sup>++</sup> (10 mL/T75). Add 0.05% trypsin/0.2 g/L EDTA (1 mL/T75) and place in humidified incubator at 37°C with 5% CO<sub>2</sub> until cells begin to round up and detach (2-4 minutes). Gently rap the side of the flask to dislodge the cells. Neutralize trypsin by addition of 4 mL Chem-9 Aequorin Growth Media per 1 mL trypsin.
5. Cells are typically passaged 1:10 every 3-4 days. Passaging ratio may be varied according to requirements of the investigator.
6. Frozen stocks of cells should be prepared at the earliest passage possible after thawing, as follows: Count detached cells (prepared as in Step 4). Centrifuge cells at 200 x g for 5 min. Resuspend cells at  $5 \times 10^6$  cells/mL in Freezing Media (cell densities of  $2-10 \times 10^6$  are also acceptable if necessary). Dispense 1 mL aliquots into cryopreservation vials. Freeze the cells by a controlled rate process, such as in an isopropanol-jacketed container placed at -70°C overnight. Store the vials in liquid nitrogen.
7. Use of cells immediately after thawing is feasible for some cell lines and is being further validated. Some cell lines may need to be passaged at least once after thawing prior to use in calcium flux assays.

**MEDIA:**

Chem-9 Aequorin Growth 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)  
250 µg/mL Genetecin/G-418  
250 µg/mL Hygromycin  
500 µg/mL Zeocin

Chem-9 Aequorin Plating Media:  
DMEM with 4.5 g/L glucose and 4 mM glutamine  
10% heat-inactivated FBS  
1x NEAA  
10mM HEPES  
1x Pen-Strep

Freezing Media:  
90% heat-inactivated FBS  
10% DMSO (cell culture grade)

**RECOMMENDED  
ASSAY CONDITIONS:**

1. Seed cells in 96-well white plate (top-read instruments) or opaque walled, clear bottom plate (bottom-read instruments) overnight at 50,000 cells/well in Chem-9 Aequorin Plating Media.
2. Wash cells once (200  $\mu$ l/well) with Wash Buffer (HBSS with  $Ca^{++}$  and  $Mg^{++}$  containing 10 mM HEPES) before loading with 5 $\mu$ M of coelenterazine (Millipore ES016) in wash buffer at room temperature for 3 hours.

**Note:** *Luminescence activity has been determined to be optimal at room temperature. Incubation at 37 °C will result in substantially reduced signals.*

3. After loading, wash cells once with Wash Buffer (200  $\mu$ l/well) prior to addition of ligands.

**REFERENCE:**

Kennedy CR *et al.* (1999) Salt-sensitivity hypertension and reduced fertility in mice lacking the prostaglandin EP<sub>2</sub> receptor. *Nat. Med.* 5:217-220.

Narumiya S and FitzGerald GA (2001) Genetic and pharmacological analysis of prostanoid receptor function. *J. Clin. Invest.* 108: 25-30.

Yang N *et al.* (2003) Cancer-associated immunodeficiency and dendritic cell abnormalities mediated by the prostaglandin EP<sub>2</sub> receptor. *J. Clin. Invest.* 111: 727-735.

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

**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.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

©2008: Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission 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  
www.millipore.com

# MILLIPORE

**CHEMICON**  
now part of Millipore

**User Agreement (Label License) for ChemiScreen™ FLASH AEQUORIN CALCIUM-OPTIMIZED STABLE CELL LINE  
HUMAN RECOMBINANT EP<sub>2</sub> PROSTANOID RECEPTOR****Product No. HTS185AF**

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 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

# MILLIPORE

# CHEMICON

---

now part of Millipore

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.