

## PRODUCT DATASHEET

### Ready-to-Assay™ PTH<sub>2</sub> Parathyroid Hormone Receptor Frozen Cells

#### CATALOG NUMBER: HTS173RTA

**CONTENTS:** Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

**STORAGE:** Vials are to be stored in liquid N<sub>2</sub>. Media Component at 4°C (-20°C for prolonged storage).

#### BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

Parathyroid hormone (PTH) is recognized by three different class B GPCRs; PTH<sub>1</sub>, PTH<sub>2</sub>, and PTH<sub>3</sub>, which couple to Gs to stimulate cAMP production. The PTH<sub>1</sub> receptor is found in high levels in the kidney and bone, where it binds PTH and PTHrP to regulate Ca<sup>2+</sup> homeostasis (Brown *et al*, 1996). The PTH<sub>2</sub> receptor, which binds PTH and the neuropeptide tuberoinfundibular peptide 39 (TIP-39) but not PTHrP, has about 50% amino acid sequence identity to the PTH<sub>1</sub> receptor. PTH<sub>2</sub> is found in the greatest volume in the nervous system and at a very low density in the kidney and bone (Usdin *et al*, 1995). The PTH<sub>3</sub> receptor, which has been recently discovered in the zebrafish, has 61% amino acid identity to the zebrafish PTH<sub>1</sub> receptor and also seems to share its ligand affinity (Rubin *et al*, 1999). Studies with the selective PTH<sub>2</sub> receptor agonist, TIP-39, and the distribution of the receptor in the superficial dorsal horn of the spinal cord suggest the receptor may play a role in pain perception (Usdin *et al*, 1999). Cloned human PTH<sub>2</sub>-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PTH<sub>2</sub> expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at PTH<sub>2</sub>.

#### USE RESTRICTIONS

Please see User Agreement (Label License) for further details. **One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.**

#### WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures  
Not for Animal or Human Consumption

#### 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.

## APPLICATIONS

Calcium Flux Assays, cAMP accumulation

### APPLICATION DATA

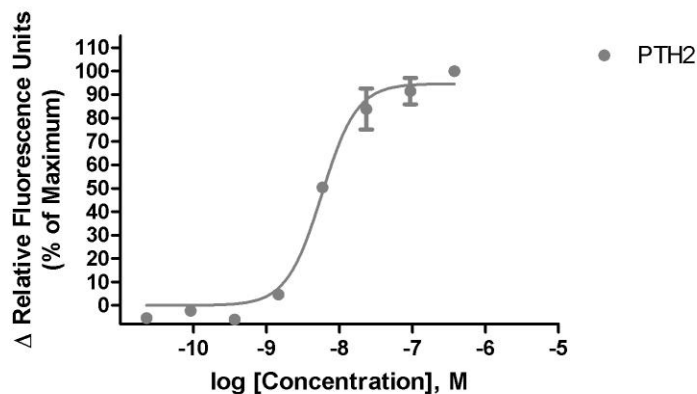


Figure 1. Representative data for activation of PTH<sub>2</sub> receptor. Calcium flux in PTH<sub>2</sub>-expressing Chem-1 cell line induced by TIP-39. PTH<sub>2</sub>-expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR<sup>TETRA</sup>. Maximal fluorescence signal obtained in this experiment was 400 RLU (Relative Light Units).

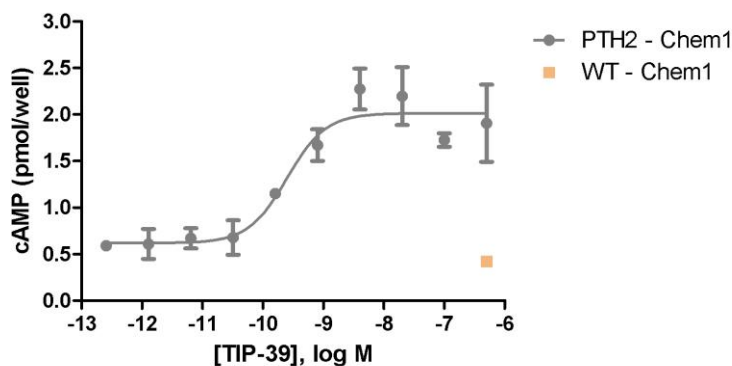


Figure 2. Representative data for activation of PTH<sub>2</sub> receptor stably expressed in Chem-1 cells induced by TIP-39 using a cAMP accumulation assay. PTH<sub>2</sub>-expressing Chem-1 cells were seeded at 100,000 cells per well into a 96-well plate, and the following day the cells were treated with TIP-39 for 15 minutes in the presence of 2.0 mM IBMX and 0.5% DMSO to determine receptor-mediated cAMP generation using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay measured on the BioTek Synergy. Maximal cAMP response obtained in this experiment was 3 pmol/well. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. Comparison of EC<sub>50</sub> values of PTH<sub>2</sub>-expressing Chem-1 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
TIP-29	Calcium Flux	6	Eurofins Internal Data
TIP-39	cAMP	0.3	Eurofins Internal Data

## ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
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.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37°C 5% CO<sub>2</sub> incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca<sup>2+</sup> dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca<sup>2+</sup> dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca<sup>2+</sup> dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR<sup>TETRA</sup>) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR<sup>TETRA</sup>) or emission filter for Ca<sup>2+</sup> dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

## ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenecid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
TIP-39 ligand	Anaspec: 21687
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

## FLIPR SETTINGS

Settings for FLIPR<sup>TETRA</sup>® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

## HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

## EXONGENOUS GENE EXPRESSION

PTHR2 cDNA (Accession Number: NM\_005048; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

## CODING SEQUENCE

```

ATG GCC GGG CTG GGG GCG TCG CTC CAC GTC TGG GGT TGG CTA ATG CTC GGC AGC      54
M  A  G  L  G  A  S  L  H  V  W  G  W  L  M  L  G  S      18
TGC CTC CTG GCC AGA GCC CAG CTG GAT TCT GAT GGC ACC ATT ACT ATA GAG GAG      108
C  L  L  A  R  A  Q  L  D  S  D  G  T  I  T  I  E  E      36
CAG ATT GTC CTT GTG CTG AAA GCG AAA GTA CAA TGT GAA CTC AAC ATC ACA GCT      162
Q  I  V  L  V  L  K  A  K  V  Q  C  E  L  N  I  T  A      54
CAA CTC CAG GAG GGA GAA GGT AAT TGT TTC CCT GAA TGG GAT GGA CTC ATT TGT      216
Q  L  Q  E  G  E  G  N  C  F  P  E  W  D  G  L  I  C      72
TGG CCC AGA GGA ACA GTG GGG AAA ATA TCG GCT GTT CCA TGC CCT CCT TAT ATT      270
W  P  R  G  T  V  G  K  I  S  A  V  P  C  P  P  Y  I      90
TAT GAC TTC AAC CAT AAA GGA GTT GCT TTC CGA CAC TGT AAC CCC AAT GGA ACA      324
Y  D  F  N  H  K  G  V  A  F  R  H  C  N  P  N  G  T      108
TGG GAT TTT ATG CAC AGC TTA AAT AAA ACA TGG GCC AAT TAT TCA GAC TGC CTT      378
W  D  F  M  H  S  L  N  K  T  W  A  N  Y  S  D  C  L      126
CGC TTT CTG CAG CCA GAT ATC AGC ATA GGA AAG CAA GAA TTC TTT GAA CGC CTC      432
R  F  L  Q  P  D  I  S  I  G  K  Q  E  F  F  E  R  L      144
TAT GTA ATG TAT ACC GTT GGC TAC TCC ATC TCT TTT GGT TCC TTG GCT GTG GCT      486
Y  V  M  Y  T  V  G  Y  S  I  S  F  G  S  L  A  V  A      162
ATT CTC ATC ATT GGT TAC TTC AGA CGA TTG CAT TGC ACT AGG AAC TAT ATC CAC      540
I  L  I  I  G  Y  F  R  R  L  H  C  T  R  N  Y  I  H      180
ATG CAC TTA TTT GTG TCT TTC ATG CTG AGA GCT ACA AGC ATC TTT GTC AAA GAC      594
M  H  L  F  V  S  F  M  L  R  A  T  S  I  F  V  K  D      198
AGA GTA GTC CAT GCT CAC ATA GGA GTA AAG GAG CTG GAG TCC CTA ATA ATG CAG      648
R  V  V  H  A  H  I  G  V  K  E  L  E  S  L  I  M  Q      216
GAT GAC CCA CAA AAT TCC ATT GAG GCA ACT TCT GTG GAC AAA TCA CAA TAT ATC      702
D  D  P  Q  N  S  I  E  A  T  S  V  D  K  S  Q  Y  I      234

```

```

GGG TGC AAG ATT GCT GTT GTG ATG TTT ATT TAC TTC CTG GCT ACA AAT TAT TAT 756
G C K I A V V M F I Y F L A T N Y Y 252
TGG ATC CTG GTG GAA GGT CTC TAC CTG CAT AAT CTC ATC TTT GTG GCT TTC TTT 810
W I L V E G L Y L H N L I F V A F F 270
TCG GAC ACC AAA TAC CTG TGG GGC TTC ATC TTG ATA GGC TGG GGG TTT CCA GCA 864
S D T K Y L W G F I L I G W G F P A 288
GCA TTT GTT GCA GCA TGG GCT GTG GCA CGA GCA ACT CTG GCT GAT GCG AGG TGC 918
A F V A A W A V A R A T L A D A R C 306
TGG GAA CTT AGT GCT GGA GAC ATC AAG TGG ATT TAT CAA GCA CCG ATC TTA GCA 972
W E L S A G D I K W I Y Q A P I L A 324
GCT ATT GGG CTG AAT TTT ATT CTG TTT CTG AAT ACG GTT AGA GTT CTA GCT ACC 1026
A I G L N F I L F L N T V R V L A T 342
AAA ATC TGG GAG ACC AAT GCA GTT GGG CAT GAC ACA AGG AAG CAA TAC AGG AAA 1080
K I W E T N A V G H D T R K Q Y R K 360
CTG GCC AAA TCG ACA CTG GTC CTG GTC CTA GTC TTT GGA GTG CAT TAC ATC GTG 1134
L A K S T L V L V L V F G V H Y I V 378
TTC GTA TGC CTG CCT CAC TCC TTC ACT GGG CTC GGG TGG GAG ATC CGC ATG CAC 1188
F V C L P H S F T G L G W E I R M H 396
TGT GAG CTC TTC TTC AAC TCC TTT CAG GGT TTC TTT GTG TCT ATC ATC TAC TGC 1242
C E L F F N S F Q G F F V S I I Y C 414
TAC TGC AAT GGA GAG GTT CAG GCA GAG GTG AAG AAG ATG TGG AGT CGG TGG AAC 1296
Y C N G E V Q A E V K K M W S R W N 432
CTC TCC GTG GAC TGG AAA AGG ACA CCG CCA TGT GGC AGC CGC AGA TGC GGC TCA 1350
L S V D W K R T P C G S R R C G S 450
GTG CTC ACC ACC GTG ACG CAC AGC ACC AGC AGC CAG TCA CAG GTG GCG GCC AGC 1404
V L T T V T H S T S S Q S Q V A A S 468
ACA CGC ATG GTG CTT ATC TCT GGC AAA GCT GCC AAG ATC GCC AGC AGA CAG CCT 1458
T R M V L I S G K A A K I A S R Q P 486
GAC AGC CAC ATC ACT TTA CCT GGC TAT GTC TGG AGT AAC TCA GAG CAG GAC TGC 1512
D S H I T L P G Y V W S N S E Q D C 504
CTG CCA CAC TCT TTC CAC GAG GAG ACC AAG GAA GAT AGT GGG AGG CAG GGA GAT 1566
L P H S F H E E T K E D S G R Q G D 522
GAT ATT CTA ATG GAG AAG CCT TCC AGG CCT ATG GAA TCT AAC CCA GAC ACT GAA 1620
D I L M E K P S R P M E S N P D T E 540

GGA TGC CAA GGA GAA ACT GAG GAT GTT CTC TGA
G C Q G E T E D V L Stp

```

## RELATED PRODUCTS

### PRODUCT NUMBER

### DESCRIPTION

**HTSCHEM-1RTA**

Ready-to-Assay™ Chem-1 host frozen cells (control cells)

**HTS173M**

ChemiScreen™ PTH2 Parathyroid Hormone receptor membrane prep

## REFERENCES

1. Brown EM *et al.*, (1996) Serpentine receptors for parathyroid hormone, calcitonin and extracellular calcium ions. *Bailliere's Clin. Endocrinol. Metab.* 10:123–161.
2. Usdin, TB *et al.*, (1995) The PTH<sub>2</sub> receptor and TIP39: a new peptide–receptor system *J. Biol. Chem.* 270: 15455-15458.
3. Rubin DA *et al.*, (1999) Zebrafish express the common parathyroid hormone/parathyroid hormone-related peptide receptor (PTH1R) and a novel receptor (PTH3R) that is preferentially activated by mammalian and fugu fish parathyroid hormone-related peptide. *J. Biol. Chem.* 274:28185–28190.
4. Usdin, TB *et al.*, (1999) TIP39: a new neuropeptide and PTH2-receptor agonist from hypothalamus. *Nat Neurosci.* 2: 941-943

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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.

No part of these works may be reproduced in any form without permission in writing.

## User Agreement (Label License)

In addition to the General Terms and Conditions section, these specific terms also apply for **Ready-to-Assay™ PTH<sub>2</sub> Parathyroid Hormone Receptor Frozen Cells, Product No. HTS173RTA**

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 Eurofins Pharma Bioanalytics Services US Inc. 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 Eurofins Pharma Bioanalytics Services US Inc. distributor, Licensee shall be obligated to disclose its identity to Eurofins Pharma Bioanalytics Services US Inc. to insure compliance with this User Agreement.

**Limited License and Restrictions.** Pursuant to the terms and conditions of this Agreement, Eurofins Pharma Bioanalytics Services US Inc. 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 and/or re-frozen 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 Eurofins Pharma Bioanalytics Services US Inc. that Licensee will properly test and use any Product purchased from Eurofins Pharma Bioanalytics Services US Inc. 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 Eurofins Pharma Bioanalytics Services US Inc. 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 Eurofins Pharma Bioanalytics Services US Inc. 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.

**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. Eurofins Pharma Bioanalytics Services US Inc. 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 Eurofins Pharma Bioanalytics Services US Inc.'s option, return or destroy all Products (certifying such destruction to Eurofins Pharma Bioanalytics Services US Inc. 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 Eurofins Pharma Bioanalytics Services US Inc.. Any attempted assignment, sublicense or transfer by Licensee without such consent shall be null and void.

**Eurofins Pharma Bioanalytics Services US Inc.** is an independent member of Eurofins Discovery Services