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PRODUCT DATASHEET

Ready-to-Assay™ PRP Prolactin-Releasing Peptide Family Receptor Frozen Cells

CATALOG NUMBER: HTS057RTA

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_2. Media Component at 4ºC (-20ºC 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.

PRP, encoded by the GPR10 (or hGR3) gene, is a Gq-coupled receptor for prolactin-releasing peptide that is expressed in the pituitary (Hinuma et al., 1998). Genetic studies in rodents indicate that lack of GPR10 leads to hyperphagia, obesity and dyslipidemia (Gu et al., 2004; Watanabe et al., 2005). In humans, genetic variations in GPR10 are associated with lowered blood pressure (Bhattacharyya et al., 2003). Cloned human PRP-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant PRP expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at PRP.

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.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

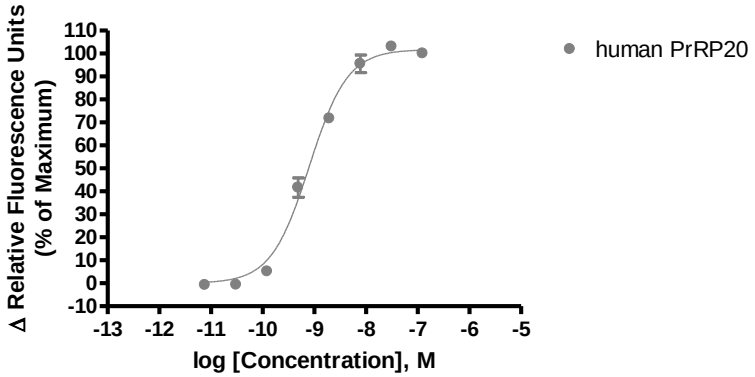

Figure 1. Representative data for activation of PRP receptor. Calcium flux in PRP –expressing Chem-1 cell line induced by human Prolactin-releasing Peptide Fragment 12-31 (synonyms: PrP-20, PrRP20). PRP –expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,500 RLU (Relative Light Units).

Table 1. EC_{50} value of PRP -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Human Prolactin-releasing Peptide Fragment 12-31	Calcium Flux	0.8	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 37ºC 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 37ºC 5% CO2 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^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} 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 ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm ($FLIPR^{TETRA}$) or emission filter for Ca^{2+} 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
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
PrP-20 ligand	Sigma: P7107
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 $FLIPR^{TETRA}$® 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

PRLHR / GPR10 cDNA (Accession Number: NM_004248; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                                        ATG GCC TCA TCG ACC ACT     60
                                                         M   A   S   S   T   T     20

CGG GGC CCC AGG GTT TCT GAC TTA TTT TCT GGG CTG CCG CCG GCG GTC ACA ACT CCC GCC    120
 R   G   P   R   V   S   D   L   F   S   G   L   P   P   A   V   T   T   P   A     40

AAC CAG AGC GCA GAG GCC TCG GCG GGC AAC GGG TCG GTG GCT GGC GCG GAC GCT CCA GCC    180
 N   Q   S   A   E   A   S   A   G   N   G   S   V   A   G   A   D   A   P   A     60

GTC ACG CCC TTC CAG AGC CTG CAG CTG GTG CAT CAG CTG AAG GGG CTG ATC GTG TTG CTC    240
 V   T   P   F   Q   S   L   Q   L   V   H   Q   L   K   G   L   I   V   L   L     80

TAC AGC GTC GTG GTG GTC GTG GGG CTG GTG GGC AAC TGC CTG CTG GTG CTG GTG ATC GCG    300
 Y   S   V   V   V   V   V   G   L   V   G   N   C   L   L   V   L   V   I   A    100

CGG GTG CGC CGG CTG CAC AAC GTG ACG AAC TTC CTC ATC GGC AAC CTG GCC TTG TCC GAC    360
 R   V   R   R   L   H   N   V   T   N   F   L   I   G   N   L   A   L   S   D    120

GTG CTC ATG TGC ACC GCC TGC GTG CCG CTC ACG CTG GCC TAT GCC TTC GAG CCA CGC GGC    420
 V   L   M   C   T   A   C   V   P   L   T   L   A   Y   A   F   E   P   R   G    140

TGG GTG TTC GGC GGC GGC CTG TGC CAC CTG GTC TTC TTC CTG CAG CCG GTC ACC GTC TAT    480
 W   V   F   G   G   G   L   C   H   L   V   F   F   L   Q   P   V   T   V   Y    160

GTG TCG GTG TTC ACG CTC ACC ACC ATC GCA GTG GAC CGC TAC GTC GTG CTG GTG CAC CCG    540
 V   S   V   F   T   L   T   T   I   A   V   D   R   Y   V   V   L   V   H   P    180

CTG AGG CGG CGC ATC TCG CTG CGC CTC AGC GCC TAC GCT GTG CTG GCC ATC TGG GCG CTG    600
 L   R   R   R   I   S   L   R   L   S   A   Y   A   V   L   A   I   W   A   L    200

TCC GCG GTG CTG GCG CTG CCC GCC GCC GTG CAC ACC TAT CAC GTG GAG CTC AAG CCG CAC    660
 S   A   V   L   A   L   P   A   A   V   H   T   Y   H   V   E   L   K   P   H    220

GAC GTG CGC CTC TGC GAG GAG TTC TGG GGC TCC CAG GAG CGC CAG CGC CAG CTC TAC GCC    720
 D   V   R   L   C   E   E   F   W   G   S   Q   E   R   Q   R   Q   L   Y   A    240

TGG GGG CTG CTG CTG GTC ACC TAC CTG CTC CCT CTG CTG GTC ATC CTC CTG TCT TAC GTC    780
 W   G   L   L   L   V   T   Y   L   L   P   L   L   V   I   L   L   S   Y   V    260

CGG GTG TCA GTG AAG CTC CGC AAC CGC GTG GTG CCG GGC TGC GTG ACC CAG AGC CAG GCC    840
 R   V   S   V   K   L   R   N   R   V   V   P   G   C   V   T   Q   S   Q   A    280

GAC TGG GAC CGC GCT CGG CGC CGG CGC ACC TTC TGC TTG CTG GTG GTG GTC GTG GTG GTG    900
 D   W   D   R   A   R   R   R   R   T   F   C   L   L   V   V   V   V   V   V    300

TTC GCC GTC TGC TGG CTG CCG CTG CAC GTC TTC AAC CTG CTG CGG GAC CTC GAC CCC CAC    960
 F   A   V   C   W   L   P   L   H   V   F   N   L   L   R   D   L   D   P   H    320

GCC ATC GAC CCT TAC GCC TTT GGG CTG GTG CAG CTG CTC TGC CAC TGG CTC GCC ATG AGT   1020
 A   I   D   P   Y   A   F   G   L   V   Q   L   L   C   H   W   L   A   M   S    340

TCG GCC TGC TAC AAC CCC TTC ATC TAC GCC TGG CTG CAC GAC AGC TTC CGC GAG GAG CTG   1080
 S   A   C   Y   N   P   F   I   Y   A   W   L   H   D   S   F   R   E   E   L    360

CGC AAA CTG TTG GTC GCT TGG CCC CGC AAG ATA GCC CCC CAT GGC CAG AAT ATG ACC GTC   1140
 R   K   L   L   V   A   W   P   R   K   I   A   P   H   G   Q   N   M   T   V    380

AGC GTG GTC ATC TGA
 S   V   V   I  Stp
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS057M	ChemiScreen™ PRP prolactin-releasing peptide family receptor membrane prep

REFERENCES

1. Bhattacharyya S *et al.* (2003) Association of polymorphisms in GPR10, the gene encoding the prolactin-releasing peptide receptor with blood pressure, but not obesity, in a U.K. Caucasian population. *Diabetes* 52: 1296-9.
2. Gu W *et al.* (2004) The prolactin-releasing peptide receptor (GPR10) regulates body weight homeostasis in mice. *J. Mol. Neurosci.* 22: 93-103.
3. Hinuma S *et al.* (1998) A prolactin-releasing peptide in the brain. *Nature* 393: 272-6.
4. Watanabe TK *et al.* (2005) Mutated G-protein-coupled receptor GPR10 is responsible for the hyperphagia/dyslipidaemia/obesity locus of Dmo1 in the OLETF rat. *Clin. Exp. Pharmacol. Physiol.* 32: 355-66.

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