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PRODUCT DATASHEET

ChemiScreen™ PRP/GPR10 Prolactin-Releasing Peptide Receptor Stable Cell Line

CATALOG NUMBER: HTS057C

CONTENTS: 2 vials of mycoplasma-free cells, 1 mL per vial.

STORAGE: Vials are to be stored in liquid N_2.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

PRP, encoded by the GPR10 (or hGR3) gene, is a G_q-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). The 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 and contains high levels of the promiscuous G protein $G_{\alpha 15}$ to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between PRP and its ligands.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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 Fluorescence Assay

APPLICATION DATA

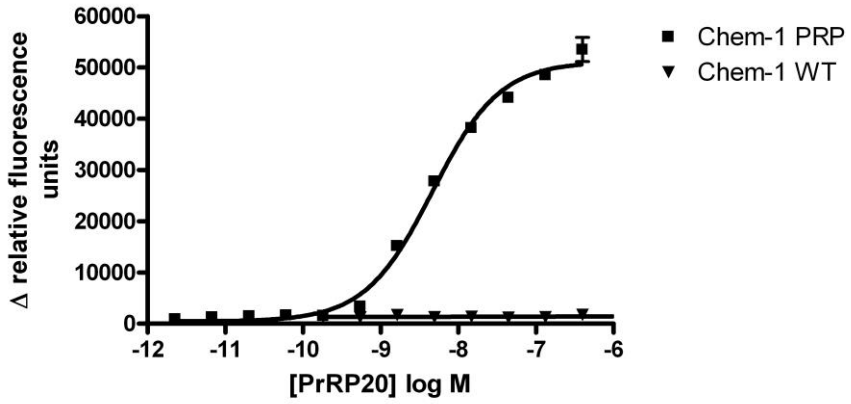

Figure 1. Representative data for activation of the PRP receptor stably expressed in Chem-1 cells induced by PrP-20 using a fluorescent calcium flux assay. PRP–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 9,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
PrP-20	Calcium Flux - Fluorescence	5	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH30070.03
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH30238.01
	HEPES	1X	EMD Millipore: TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
Dissociation	Sterile PBS	-	Hyclone: SH30028.03
	0.25% Trypsin-EDTA	-	Hyclone: SH30042.01
CryoMedium	Basal Medium (see above)	40%	
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH30070.03
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP

Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
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 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712
Coelenterazine-h (250µg). Prepare to 10mM	Promega: S2011

Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
6. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
11. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

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

EXOGENOUS GENE EXPRESSION

Human PRP cDNA (Accession Number: NM_004248; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

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-1	ChemiScreen™ Chem-1 Parental Cell Line (control cells)
HTS057M	ChemiScreen™ PRP/GPR10 Prolactin-Releasing Peptide 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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