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PRODUCT DATASHEET

ChemiScreen™ $NPBW_1$ Neuropeptide B/W Receptor Stable Cell Line

CATALOG NUMBER: HTS180C

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-4 host, which supports high levels of functional receptor expression on the cell surface. Chem-4 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Neuropeptide B (NPB) and neuropeptide W (NPW) are members of a recently identified neuropeptide family that are ligands for two highly similar receptors, $NPBW_1$ (GPR7) and $NPBW_2$ (GPR8), both of which couple to Gi/o to inhibit intracellular cAMP production. Highest expression of $NPBW_1$ mRNA and protein was identified in the amygdala and hypothalamic nuclei. Physiological studies demonstrate that intracerebroventricular infusion of $NPBW_1$ ligands modulates feeding behaviour, nociception, and release of corticosterone, prolactin and growth hormone (Singh and Davenport, 2006). $NPBW_1$ knock out male mice have shown mild adult-onset obesity and decreased locomotor activity. They become progressively hyperglycaemic and hyperinsulinaemic (Ishii *et al*., 2003). The cloned human $NPBW_1$-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant $NPBW_1$ expression on the cell surface and contains high levels of the promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for antagonists of interactions between $NPBW_1$ 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

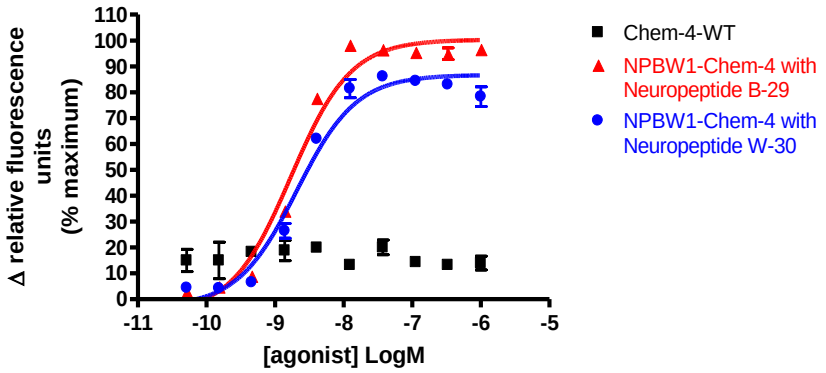

Figure 1. Representative data for activation of the $NPBW_1$ receptor stably expressed in Chem-4 cells induced by Neuropeptide B-29 using a fluorescent calcium flux assay. $NPBW_1$–expressing Chem-4 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were 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 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-4) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} value of $NPBW_1$-expressing Chem-4 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
Neuropeptide B-29	Calcium Flux - Fluorescence	2.0	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
	Hygromycin	250 µg/ml	Invivogen: ant-hg-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	0.60	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
Neuropeptide B-29 ligand	Phoenix: 005-51
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.

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HOST CELL

Chem-4, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS GENE EXPRESSION

Human NPBW1 cDNA (Accession Number: NM_005285; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

CODING SEQUENCE

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ATG GAC AAC GCC TCG TTC TCG GAG CCC TGG CCC GCC AAC GCA TCG GGC CCG GAC CCG GCG CTG AGC TGC     69
 M   D   N   A   S   F   S   E   P   W   P   A   N   A   S   G   P   D   P   A   L   S   C     23

TCC AAC GCG TCG ACT CTG GCG CCG CTG CCG GCG CCG CTG GCG GTG GCT GTA CCA GTT GTC TAC GCG GTG    138
 S   N   A   S   T   L   A   P   L   P   A   P   L   A   V   A   V   P   V   V   Y   A   V     46

ATC TGC GCC GTG GGT CTG GCG GGC AAC TCC GCC GTG CTG TAC GTG TTG CTG CGG GCG CCC CGC ATG AAG    207
 I   C   A   V   G   L   A   G   N   S   A   V   L   Y   V   L   L   R   A   P   R   M   K     69

ACC GTC ACC AAC CTG TTC ATC CTC AAC CTG GCC ATC GCC GAC GAG CTC TTC ACG CTG GTG CTG CCC ATC    276
 T   V   T   N   L   F   I   L   N   L   A   I   A   D   E   L   F   T   L   V   L   P   I     92

AAC ATC GCC GAC TTC CTG CTG CGG CAG TGG CCC TTC GGG GAG CTC ATG TGC AAG CTC ATC GTG GCT ATC    345
 N   I   A   D   F   L   L   R   Q   W   P   F   G   E   L   M   C   K   L   I   V   A   I    115

GAC CAG TAC AAC ACC TTC TCC AGC CTC TAC TTC CTC ACC GTC ATG AGC GCC GAC CGC TAC CTG GTG GTG    414
 D   Q   Y   N   T   F   S   S   L   Y   F   L   T   V   M   S   A   D   R   Y   L   V   V    138

TTG GCC ACT GCG GAG TCG CGC CGG GTG GCC GGC CGC ACC TAC AGC GCC GCG CGC GCG GTG AGC CTG GCC    483
 L   A   T   A   E   S   R   R   V   A   G   R   T   Y   S   A   A   R   A   V   S   L   A    161

GTG TGG GGG ATC GTC ACA CTC GTC GTG CTG CCC TTC GCA GTC TTC GCC CGG CTA GAC GAC GAG CAG GGC    552
 V   W   G   I   V   T   L   V   V   L   P   F   A   V   F   A   R   L   D   D   E   Q   G    184

CGG CGC CAG TGC GTG CTA GTC TTT CCG CAG CCC GAG GCC TTC TGG TGG CGC GCG AGC CGC CTC TAC ACG    621
 R   R   Q   C   V   L   V   F   P   Q   P   E   A   F   W   W   R   A   S   R   L   Y   T    207

CTC GTG CTG GGC TTC GCC ATC CCC GTG TCC ACC ATC TGT GTC CTC TAT ACC ACC CTG CTG TGC CGG CTG    690
 L   V   L   G   F   A   I   P   V   S   T   I   C   V   L   Y   T   T   L   L   C   R   L    230

CAT GCC ATG CGG CTG GAC AGC CAC GCC AAG GCC CTG GAG CGC GCC AAG AAG CGG GTG ACC TTC CTG GTG    759
 H   A   M   R   L   D   S   H   A   K   A   L   E   R   A   K   K   R   V   T   F   L   V    253

GTG GCA ATC CTG GCG GTG TGC CTC CTC TGC TGG ACG CCC TAC CAC CTG AGC ACC GTG GTG GCG CTC ACC    828
 V   A   I   L   A   V   C   L   L   C   W   T   P   Y   H   L   S   T   V   V   A   L   T    276

ACC GAC CTC CCG CAG ACG CCG CTG GTC ATC GCT ATC TCC TAC TTC ATC ACC AGC CTG ACG TAC GCC AAC    897
 T   D   L   P   Q   T   P   L   V   I   A   I   S   Y   F   I   T   S   L   T   Y   A   N    299

AGC TGC CTC AAC CCC TTC CTC TAC GCC TTC CTG GAC GCC AGC TTC CGC AGG AAC CTC CGC CAG CTG ATA    966
 S   C   L   N   P   F   L   Y   A   F   L   D   A   S   F   R   R   N   L   R   Q   L   I    322

ACT TGC CGC GCG GCA GCC TGA
 T   C   R   A   A   A  Stp
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RELATED PRODUCTS

Product Number	Description
HTSCHEM-4	ChemiScreen™ Chem-4 Parental Cell Line (control cells)
HTS180M	ChemiScreen™ $NPBW_1$ Neuropeptide B/W Receptor Membrane Prep

REFERENCES

1. Singh G and Davenport AP (2006) Neuropeptide B and W: neurotransmitters in an emerging G-protein-coupled receptor system. *Br. J. Pharmacol.* 148:1033-41.
2. Ishii M *et al.* (2003). Targeted disruption of GPR7, the endogenous receptor for neuropeptides B and W, leads to metabolic defects and adult-onset obesity. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10540–10545.

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