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PRODUCT DATASHEET

Ready-to-Assay™ NPBW1 Neuropeptide B/W Receptor Frozen Cells

CATALOG NUMBER: HTS180RTA

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.

Neuropeptide B (NPB) and neuropeptide W (NPW) are members of a recently identified neuropeptide family that are ligands for two highly similar receptors, NPBW1 (GPR7) and NPBW2 (GPR8), both of which couple to Gi/o to inhibit intracellular cAMP production. Highest expression of NPBW1 mRNA and protein was identified in the amygdala and hypothalamic nuclei. Physiological studies demonstrate that intracerebroventricular infusion of NPBW1 ligands modulates feeding behaviour, nociception, and release of corticosterone, prolactin and growth hormone (Singh and Davenport, 2006). NPBW1 knock out male mice have shown mild adult-onset obesity and decreased locomotor activity. They become progressively hyperglycaemic and hyperinsulinaemic (Ishii et al., 2003). Cloned human NPBW1-expressing cell line is made in the Chem-4 host, which supports high levels of recombinant NPBW1 expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein 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 NPBW1.

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

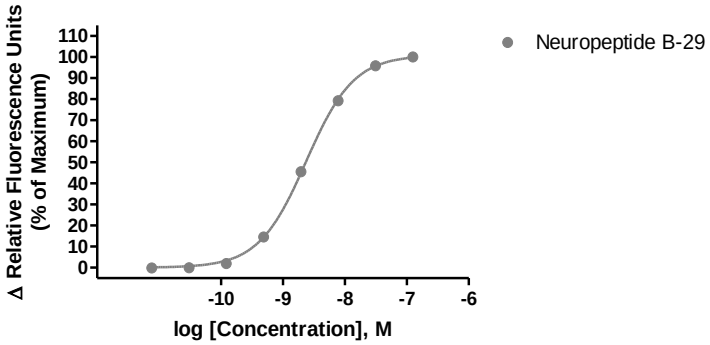

Figure 1. Representative data for activation of NPBW1 receptor. Calcium flux in NPBW1–expressing Chem-4 cell line induced by Neuropeptide B-29. NPBW1–expressing Chem-4 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 13,500 RLU (Relative Light Units).

Table 1. EC_{50} value of NPBW1-expressing Chem-4 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Neuropeptide B-29	Calcium Flux	2	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
Neuropeptide B-29 ligand	Phoenix: 005-51
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-4, an adherent rat hematopoietic cell line expressing endogenous G·15 protein as well as an exogenous proprietary promiscuous Gα protein

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EXONGENOUS GENE EXPRESSION

NPBWR1 cDNA (Accession Number: NM_005285; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

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-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS180M	ChemiScreen™ NPBW1 Neuropeptide B/W receptor membrane prep

Note: Chem-4 cells are derived from Chem-1 cells.

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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