

PRODUCT DATASHEET

Ready-to-Assay™ Y₂ Neuropeptide Y Receptor Frozen Cells

CATALOG NUMBER: HTS066RTA

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 N₂. 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.

The NPY family consists of three 36-amino acid peptides, neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP), which bind to the NPY receptor family of G protein-coupled receptors. Five NPY receptors, Y₁, Y₂, Y₄, Y₅ and Y₆, have been defined at the molecular level, and each signals primarily through G_{i/o}. Binding of NPY family peptides to NPY receptors mediates a variety of physiological effects, including promotion of food intake, decreased anxiety, inhibition of neurotransmitter and hormone release, vasoconstriction, and gut motility. Y₂ is primarily expressed in the CNS, and it mediates presynaptic inhibition of neurotransmitter release (Michel *et al.*, 1998). Millipore's cloned human Y₂-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant Y₂ 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 Y₂.

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

APPLICATION DATA

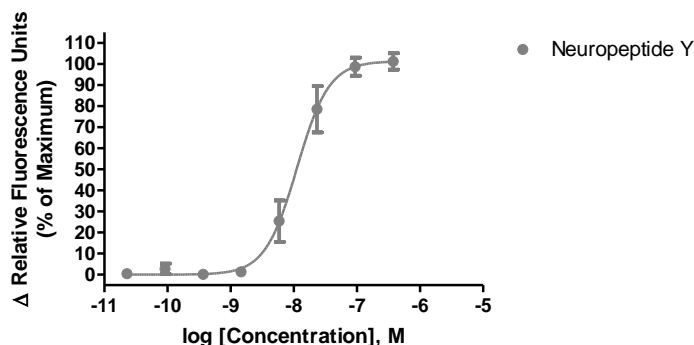


Figure 1. Representative data for activation of Y_2 receptor. Calcium flux in Y_2 -expressing Chem-1 cell line induced by Neuropeptide Y. Y_2 -expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,900 RLU (Relative Light Units).

Table 1. EC₅₀ values of Y_2 -expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Neuropeptide Y	Calcium Flux	11	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% 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) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ 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 Ca²⁺ 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 TM , AM	AAT Bioquest: 21080
Neuropeptide Y ligand	Sigma: N5017
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.

EXOGENOUS GENE EXPRESSION

NPY2R cDNA (Accession Number: NM_000910; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG GGT CCA ATA GGT GCA GAG GCT GAT GAG AAC
M G P I G A E A D E N

CAG ACA GTG GAA GAA ATG AAG GTG GAA CAA TAC GGG CCA CAA ACA ACT CCT AGA GGT GAA CTG GTC CCT
Q T V E E M K V E Q Y G P Q T T P R G E L V P

GAC CCT GAG CCA GAG CTT ATA GAT AGT ACC AAG CTG ATT GAG GTA CAA GTT GTT CTC ATA TTG GCC TAC
D P E P E L I D S T K L I E V Q V V L I L A Y

TGC TCC ATC ATC TTG CTT GGG GTA ATT GGC AAC TCC TTG GTG ATC CAT GTG GTG ATC AAA TTC AAG AGC
C S I I L L G V I G N S L V I H V V I K F K S

ATG CGC ACA GTA ACC AAC TTT TTC ATT GCC AAT CTG GCT GTG GCA GAT CTT TTG GTG AAC ACT CTG TGT
M R T V T N F F I A N L A V A D L L V N T L C

CTA CCG TTC ACT CTT ACC TAT ACC TTA ATG GGG GAG TGG AAA ATG GGT CCT GTC CTG TGC CAC CTG GTG
L P F T L T Y T L M G E W K M G P V L C H L V

CCC TAT GCC CAG GGC CTG GCA GTA CAA GTA TCC ACA ATC ACC TTG ACA GTA ATT GCC CTG GAC CGG CAC
P Y A Q G L A V Q V S T I T L T V I A L D R H

AGG TGC ATC GTC TAC CAC CTA GAG AGC AAG ATC TCC AAG CGA ATC AGC TTC CTG ATT ATT GGC TTG GCC
R C I V Y H L E S K I S K R I S F L I I G L A

TGG GGC ATC AGT GCC CTG CTG GCA AGT CCC CTG GCC ATC TTC CGG GAG TAT TCG CTG ATT GAG ATC ATT
W G I S A L L A S P L A I F R E Y S L I E I I

CCG GAC TTT GAG ATT GTG GCC TGT ACT GAA AAG TGG CCT GGC GAG GAG AAG AGC ATC TAT GGC ACT GTC
P D F E I V A C T E K W P G E E K S I Y G T V

TAT AGT CTT TCT FCC TTG TTG ATC TTG TAT GTT TTG CCT CTG GGC ATT ATA TCA TTT FCC TAC ACT CGC
Y S L S S L L I L Y V L P L G I I S F S Y T R

ATT TGG AGT AAA TTG AAG AAC CAT GTC AGT CCT GGA GCT GCA AAT GAC CAC TAC CAT CAG CGA AGG CAA
I W S K L K N H V S P G A A N D H Y H Q R R Q

AAA ACC ACC AAA ATG CTG GTG TGT GTG GTG GTG GTG TTT GCG GTC AGC TGG CTG CCT CTC CAT GCC TTC
K T T K M L V C V V V V V F A V S W L P L H A F

CAG CTT GCC GTT GAC ATT GAC AGC CAG GTC CTG GAC CTG AAG GAG TAC AAA CTC ATC TTC ACA GTG TTC
Q L A V D I D S Q V L D L K E Y K L I F T V F

CAC ATT ATC GCC ATG TGC TCC ACT TTT GCC AAT CCC CTT CTC TAT GGC TGG ATG AAC AGC AAC TAC AGA
H I I A M C S T F A N P L L Y G W M N S N Y R

AAG GCT TTC CTC TCG GCC TTC CGC TGT GAG CAG CGG TTG GAT GCC ATT CAC TCT GAG GTG TCC GTG ACA
K A F L S A F R C E Q R L D A I H S E V S V T

TTC AAG GCT AAA AAG AAC CTG GAG GTC AGA AAG AAC AGT GGC CCC AAT GAC TCT TTC ACA GAG GCT ACC
F K A K K N L E V R K N S G P N D S F T E A T

AAT GTC TGA
N V Stp

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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS160M	ChemiScreen™ Y ₂ Neuropeptide Y receptor stable cell line
HTS160M	ChemiScreen™ Y ₂ Neuropeptide Y receptor membrane prep

REFERENCES

1. Michel MC et al. (1998) XVI. International Union of Pharmacology. Recommendations for the nomenclature of neuropeptide Y, peptide YY and pancreatic polypeptide receptors. Pharmacol. Rev. 50: 143-150.

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