eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ NMU1 Neuromedin U Receptor Frozen Cells

CATALOG NUMBER: HTS062RTA

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.

Neuromedin U (NmU) is a peptide characterized by its ability to promote smooth muscle contraction (Brighton *et al.*, 2004a). Two GPCRs, NMU1 and NMU2, mediate the contractile effects of neuromedin U by activation of both G_q and G_i (Brighton *et al*., 2004b). In addition, NmU binds to NMU1 expressed on Th2 cells to induce cytokine release (Johnson *et al*., 2004). Cloned human NMU1 expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NMU1 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 NMU1.

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

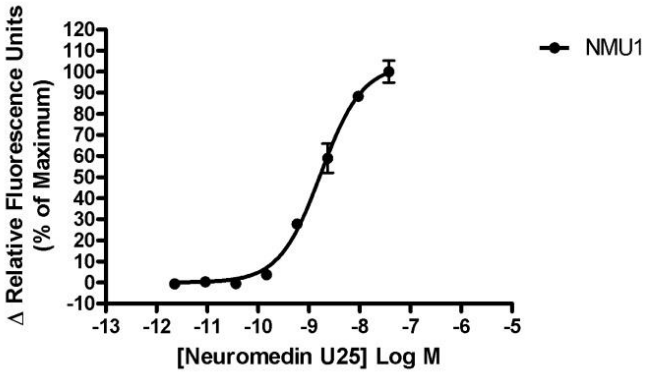

Figure 1. Representative data for activation of NMU1 receptor. Calcium flux in NMU1–expressing Chem-1 cell line induced by Neuromedin U25. NMU1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 1,100 RLU (Relative Light Units).

Table 1. EC_{50} value of NMU1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Neuromedin U25	Calcium Flux	1.7	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
Neuromedian U25 ligand	Sigma: N4284
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

NMUR1 cDNA (Accession Number: NM_006056; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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ATG ACT CCT CTC TGC CTC AAT TGC TCT GTC CTC CCT GGA GAC CTG TAC CCA GGG GGT GCA AGG AAC CCC ATG
GCT TGC AAT GGC AGT GCG GCC AGG GGG CAC TTT GAC CCT GAG GAC TTG AAC CTG ACT GAC GAG GCA CTG AGA
CTC AAG TAC CTG GGG CCC CAG CAG ACA GAG CTG TTC ATG CCC ATC TGT GCC ACA TAC CTG CTG ATC TTC GTG
GTG GGC GCT GTG GGC AAT GGG CTG ACC TGT CTG GTC ATC CTG CGC CAC AAG GCC ATG CGC ACG CCT ACC AAC
TAC TAC CTC TTC AGC CTG GCC GTG TCG GAC CTG CTG GTG CTG CTG GTG GGC CTG CCC CTG GAG CTC TAT GAG
ATG TGG CAC AAC TAC CCC TTC CTG CTG GGC GTT GGT GGC TGC TAT TTC CGC ACG CTA CTG TTT GAG ATG GTC
TGC CTG GCC TCA GTG CTC AAC GTC ACT GCC CTG AGC GTG GAA CGC TAT GTG GCC GTG GTG CAC CCA CTC CAG
GCC AGG TCC ATG GTG ACG CGG GCC CAT GTG CGC CGA GTG CTT GGG GCC GTC TGG GGT CTT GCC ATG CTC TGC
TCC CTG CCC AAC ACC AGC CTG CAC GGC ATC CAG CAG CTG CAC GTG CCC TGC CGG GGC CCA GTG CCA GAC TCA
GCT GTT TGC ATG CTG GTC CGC CCA CGG GCC CTC TAC AAC ATG GTA GTG CAG ACC ACC GCG CTG CTC TTC TTC
TGC CTG CCC ATG GCC ATC ATG AGC GTG CTC TAC CTG CTC ATT GGG CTG CGA CTG CGG CGG GAG AGG CTG CTG
CTC ATG CAG GAG GCC AAG GGC AGG GGC TCT GCA GCA GCC AGG TCC AGA TAC ACC TGC AGG CTC CAG CAG CAC
GAT CGG GGC CGG AGA CAA GTG ACC AAG ATG CTG TTT GTC CTG GTC GTG GTG TTT GGC ATC TGC TGG GCC CCG
TTC CAC GCC GAC CGC GTC ATG TGG AGC GTC GTG TCA CAG TGG ACA GAT GGC CTG CAC CTG GCC TTC CAG CAC
GTG CAC GTC ATC TCC GGC ATC TTC TTC TAC CTG GGC TCG GCG GCC AAC CCC GTG CTC TAT AGC CTC ATG TCC
AGC CGC TTC CGA GAG ACC TTC CAG GAG GCC CTG TGC CTC GGG GCC TGC TGC CAT CGC CTC AGA CCC CGC CAC
AGC TCC CAC AGC CTC AGC AGG ATG ACC ACA GGC AGC ACC CTG TGT GAT GTG GGC TCC CTG GGC AGC TGG GTC
CAC CCC CTG GCT GGG AAC GAT GGC CCA GAG GCG CAG CAA GAG ACC GAT CCA TCC TGA
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS062M	ChemiScreen™ NMU1 Neuromedin U receptor membrane prep

REFERENCES

1. Brighton PJ *et al.* (2004a) Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol. Rev.* 56: 231-48
2. Brighton PJ *et al.* (2004b) Signaling and ligand binding by recombinant neuromedin U receptors: evidence for dual coupling to Galphaq/11 and Galphai and an irreversible ligand-receptor interaction. *Mol. Pharmacol.* 66: 1544-56.
3. Johnson EN *et al.* (2004) Neuromedin U elicits cytokine release in murine Th2-type T cell clone D10.G4.1. *J. Immunol.* 173(12):7230-8

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