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# PRODUCT DATASHEET

## Ready-to-Assay™ NMU2 Neuromedin U Receptor Frozen Cells

## CATALOG NUMBER: HTS098RTA

**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 which regulates peripheral functions such as smooth muscle contraction and blood pressure, and CNS functions including nociception and feeding activity (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). Compared to the wide distribution of NMU1 in peripheral tissue, expression of NMU2 receptor is limited to areas of the brain, such as the paraventricular nucleus, along the wall of the third ventricle in the hypothalamus, the CA1 region of the hippocampus, and the spinal cord (Howard *et al.* 2000). Recent study has shown that mice deficient in NMU2 but not NMU1 receptor had impaired nociceptive responses, suggesting that the pro-nociceptive effects of NmU in mice appear to be mediated through NMU2 (Zeng *et al*., 2006; Torres *et al.* 2007). Cloned human NMU2-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant NMU2 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 NMU2.

## 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

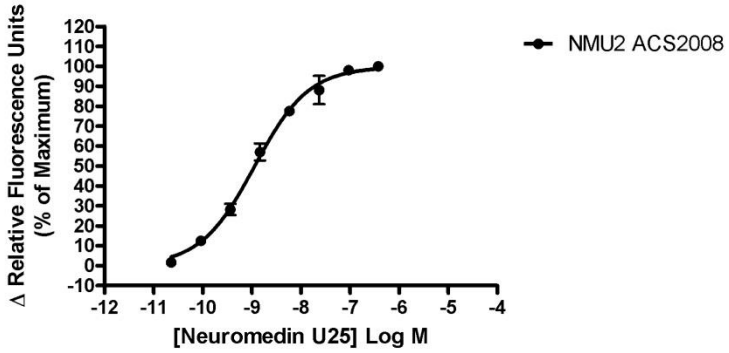


Figure 1. Representative data for activation of NMU2 receptor. Calcium flux in NMU2–expressing Chem-1 cell line induced by Neuromedin U25. NMU2–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR$^{TETRA}$. Maximal fluorescence signal obtained in this experiment was 2,000 RLU (Relative Light Units).

Table 1. $EC_{50}$ value of NMU2-expressing Chem-1 cells.

| LIGAND | ASSAY | POTENCY (nM) | REFERENCE |
|---|---|---|---|
| **Neuromedin 25** | Calcium Flux | 1 | 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 |
| Neuromedin 25 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

NMUR2 cDNA (Accession Number: NM_020167; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

## CODING SEQUENCE

```
                                                ATG TCA GGG ATG GAA AAA CTT CAG AAT GCT TCC TGG
                                                  M   S   G   M   E   K   L   Q   N   A   S   W

ATC TAC CAG CAG AAA CTA GAA GAT CCA TTC CAG AAA CAC CTG AAC AGC ACC GAG GAG TAT CTG GCC TTC
  I   Y   Q   Q   K   L   E   D   P   F   Q   K   H   L   N   S   T   E   E   Y   L   A   F

CTC TGC GGA CCT CGG CGC AGC CAC TTC TTC CTC CCC GTG TCT GTG GTG TAT GTG CCA ATT TTT GTG GTG
  L   C   G   P   R   R   S   H   F   F   L   P   V   S   V   V   Y   V   P   I   F   V   V

GGG GTC ATT GGC AAT GTC CTG GTG TGC CTG GTG ATT CTG CAG CAC CAG GCT ATG AAG ACG CCC ACC AAC
  G   V   I   G   N   V   L   V   C   L   V   I   L   Q   H   Q   A   M   K   T   P   T   N

TAC TAC CTC TTC AGC CTG GCG GTC TCT GAC CTC CTG GTC CTG CTC CTT GGA ATG CCC CTG GAG GTC TAT
  Y   Y   L   F   S   L   A   V   S   D   L   L   V   L   L   L   G   M   P   L   E   V   Y

GAG ATG TGG CGC AAC TAC CCT TTC TTG TTC GGG CCC GTG GGC TGC TAC TTC AAG ACG GCC CTC TTT GAG
  E   M   W   R   N   Y   P   F   L   F   G   P   V   G   C   Y   F   K   T   A   L   F   E

ACC GTG TGC TTC GCC TCC ATC CTC AGC ATC ACC ACC GTC AGC GTG GAG CGC TAC GTG GCC ATC CTA CAC
  T   V   C   F   A   S   I   L   S   I   T   T   V   S   V   E   R   Y   V   A   I   L   H

CCG TTC CGC GCC AAA CTG CAG AGC ACC CGG CGC CGG GCC CTC AGG ATC CTC GGC ATC GTC TGG GGC TTC
  P   F   R   A   K   L   Q   S   T   R   R   R   A   L   R   I   L   G   I   V   W   G   F

TCC GTG CTC TTC TCC CTG CCC AAC ACC AGC ATC CAT GGC ATC AAG TTC CAC TAC TTC CCC AAT GGG TCC
  S   V   L   F   S   L   P   N   T   S   I   H   G   I   K   F   H   Y   F   P   N   G   S

CTG GTC CCA GGT TCG GCC ACC TTT ACG GTC ATC AAG CCC ATG TGG ATC TAC AAT TTC ATC ATC CAG GTC
  L   V   P   G   S   A   T   F   T   V   I   K   P   M   W   I   Y   N   F   I   I   Q   V

ACC TCC TTC CTA TTC TAC CTC CTC CCC ATG ACT GTC ATC AGT GTC CTC TAC TAC CTC ATG GCA CTC AGA
  T   S   F   L   F   Y   L   L   P   M   T   V   I   S   V   L   Y   Y   L   M   A   L   R

CTA AAG AAA GAC AAA TCT CTT GAG GCA GAT GAA GGG AAT GCA AAT ATT CAA AGA CCC TGC AGA AAA TCA
  L   K   K   D   K   S   L   E   A   D   E   G   N   A   N   I   Q   R   P   C   R   K   S

GTC AAC AAG ATG CTG TTT GTC TTG GTC TTA GTG TTT GCT ATC TGT TGG GCC CCG TTC CAC ATT GAC CGA
  V   N   K   M   L   F   V   L   V   L   V   F   A   I   C   W   A   P   F   H   I   D   R

CTC TTC TTC AGC TTT GTG GAG GAG TGG AGT GAA TCC CTG GCT GCT GTG TTC AAC CTC GTC CAT GTG GTG
  L   F   F   S   F   V   E   E   W   S   E   S   L   A   A   V   F   N   L   V   H   V   V

TCA GGT GTC TTC TTC TAC CTG AGC TCA GCT GTC AAC CCC ATT ATC TAT AAC CTA CTG TCT CGC CGC TTC
  S   G   V   F   F   Y   L   S   S   A   V   N   P   I   I   Y   N   L   L   S   R   R   F

CAG GCA GCA TTC CAG AAT GTG ATC TCT TCT TTC CAC AAA CAG TGG CAC TCC CAG CAT GAC CCA CAG TTG
  Q   A   A   F   Q   N   V   I   S   S   F   H   K   Q   W   H   S   Q   H   D   P   Q   L

CCA CCT GCC CAG CGG AAC ATC TTC CTG ACA GAA TGC CAC TTT GTG GAG CTG ACC GAA GAT ATA GGT CCC
  P   P   A   Q   R   N   I   F   L   T   E   C   H   F   V   E   L   T   E   D   I   G   P

CAA TTC CCA TGT CAG TCA TCC ATG CAC AAC TCT CAC CTC CCA ACA GCC CTC TCT AGT GAA CAG ATG TCA
  Q   F   P   C   Q   S   S   M   H   N   S   H   L   P   T   A   L   S   S   E   Q   M   S

AGA ACA AAC TAT CAA AGC TTC CAC TTT AAC AAA ACC TGA
  R   T   N   Y   Q   S   F   H   F   N   K   T  Stp
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## RELATED PRODUCTS

| PRODUCT NUMBER | DESCRIPTION |
|---|---|
| **HTSCHEM-1RTA** | Ready-to-Assay™ Chem-1 host frozen cells (control cells) |
| **HTS098M** | ChemiScreen™ NMU2 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 $G_{q/11}$ and $G_i$ and an irreversible ligand-receptor interaction. *Mol. Pharmacol.* 66: 1544-56.
3. Howard AD *et al.* (2000) Identification of receptors for neuromedin U and its role in feeding. *Nature* 406: 70–74.
4. Raddatz R *et al.* (2000) Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system. *J. Biol. Chem.* 275: 32452-32459.
5. Shan LX *et al.* (2000) Identification of a novel neuromedin U receptor subtype expressed in the central nervous system. *J. Biol. Chem.* 275: 39482-39486.
6. Torres R *et al.* (2007) Mice genetically deficient in neuromedin U receptor 2, but not neuromedin U receptor 1, have impaired nociceptive responses. *Pain* 130: 267-278.
7. Zeng H *et al.* (2006) Neuromedin U receptor 2-deficient mice display differential responses in sensory perception, stress, and feeding. *Mol. Cell. Biol.* 26: 9352-9363.


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