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# PRODUCT DATASHEET

## ChemiScreen™ NMU2 Neuromedin U Receptor Stable Cell Line

## CATALOG NUMBER: HTS098C

**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-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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). The 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 and contains high levels of the promiscuous G protein to enhance coupling of the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists and antagonists at NMU2.

## 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

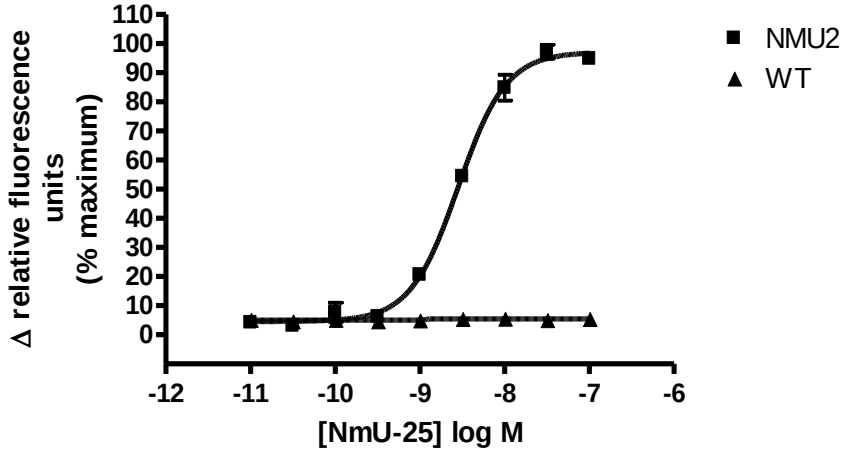


Figure 1. Representative data for activation of the NMU2 receptor stably expressed in Chem-1 cells induced by NmU-25 using a fluorescent calcium flux assay. NMU2–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent 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 6,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

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

| LIGAND | ASSAY | POTENCY $EC_{50}$ (nM) | REFERENCE |
|---|---|---|---|
| **NmU-25** | Calcium Flux - Fluorescence | 3.1 | 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 |
| **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 | 0.45 | 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 |
| NmU-25 ligand | Sigma: N4284 |
| 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.

## HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

## EXOGENOUS GENE EXPRESSION

Human NMU2 cDNA (Accession Number: NM_020167; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

## 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
```

## RELATED PRODUCTS

| Product Number | Description |
|---|---|
| **HTSCHEM-1** | ChemiScreen™ Chem-1 Parental Cell Line (control cells) |
| **HTS098M** | ChemiScreen™ NMU2 Neuromedin U Receptor Membrane Prep |

## REFERENCES


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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.
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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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