

PRODUCT DATASHEET

ChemiScreen™ NMU1 Neuromedin U Receptor Membrane Preparation

CATALOG NUMBER: HTS062M **QUANTITY:** 200 units
LOT NUMBER: JH1942127 **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

BACKGROUND: 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 Gq and Gi (Brighton *et al.*, 2004b). In addition, NmU binds to NMU1 expressed on Th2 cells to induce cytokine release (Johnson *et al.*, 2004). NMU1 membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-level of GPCR surface expression; thus, they are ideal HTS tools for screening of antagonists of NMU1 interactions with neuromedin U25. The membrane preparations exhibit a Kd of 0.08 nM for [¹²⁵I]-neuromedin U25. With 5 µg/well NMU1 Membrane Prep and 0.5 nM [¹²⁵I]-neuromedin U25, a greater than 12-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

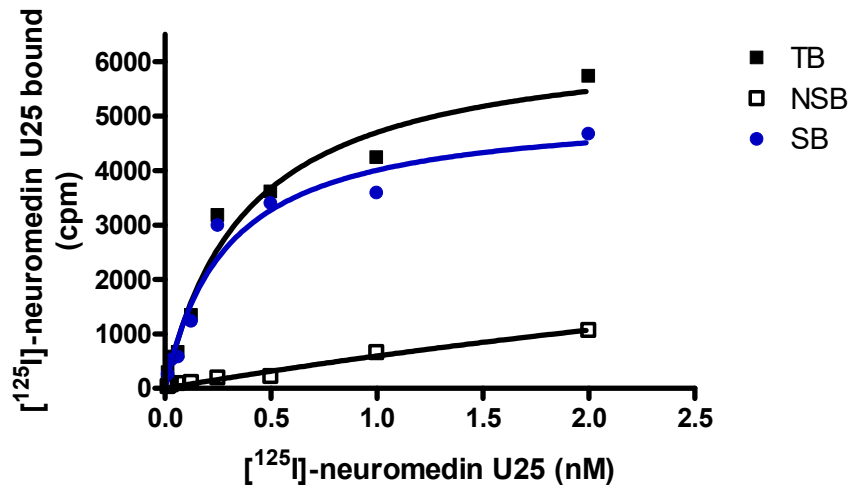


Figure 1. Saturation binding for NMU1. 5 µg/well NMU1 Membrane Preparation was incubated with increasing amount of [¹²⁵I]-neuromedin U25 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled neuromedin U25. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

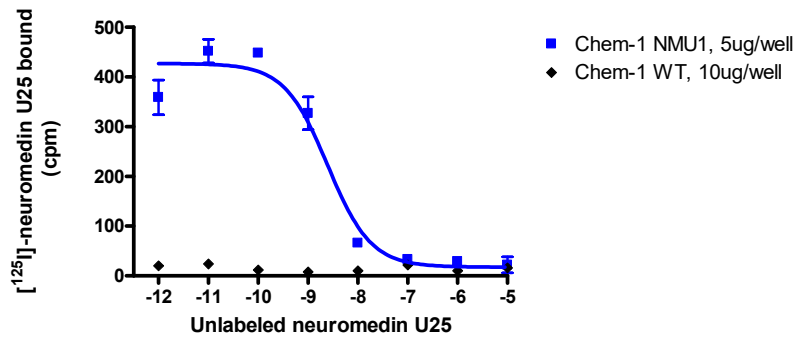


Figure 2. Competition binding for NMU1. NMU1 Membrane Preparation (5 or 10 $\mu\text{g}/\text{well}$) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.05 nM [^{125}I]-neuromedin U25 and increasing concentrations of unlabeled neuromedin U25, and more than 12- fold signal:background was obtained. Representative sample data.

SPECIFICATIONS: 1 unit = 5 μg
 B_{max} for [^{125}I]-Neuromedin U-25 binding: 8.314 pmol/mg
 K_d for [^{125}I]-Neuromedin U-25 binding: 0.42 nM
Signal:background: >12-fold

TRANSFECTION: Human NMU1 (Accession number NM_006056)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NMU1 expression.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM Hepes, pH 7.4, 5 mM MgCl_2 , 1 mM CaCl_2 , 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{125}I]-neuromedin U25 (Perkin Elmer # NEX383)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl, 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 12-fold signal:background with [^{125}I]-labeled neuromedin U25 at 0.5 nM.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.

Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at -70°C . Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

- 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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