

CHEMISCREEN[™] MEMBRANE PREPARATION RECOMBINANT HUMAN NMU1 NEUROMEDIN RECEPTOR

CATALOG NUMBER: HTS062M **QUANTITY:** 200 units
LOT NUMBER: **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). Chemicon's 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 K_d 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, and GTPγS binding.

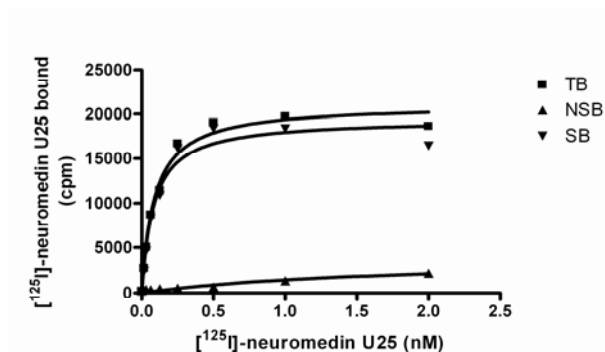


Figure 1. Saturation binding for NMU1. 5 μg/well NMU1 Membrane Preparation was incubated with increasing amount of [¹²⁵I]-neuromedin U25 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled neuromedin U25. Specific binding (SB) was determined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

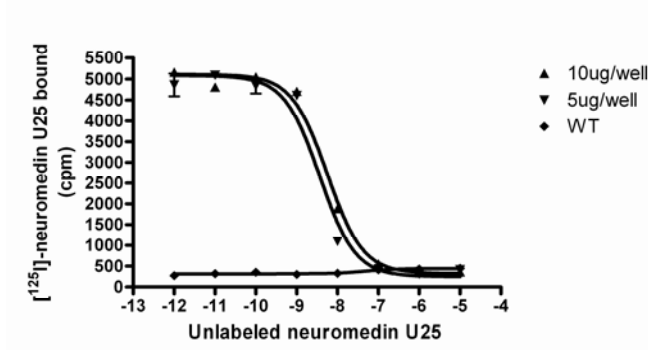


Figure 2. Competition binding for NMU1. NMU1 Membrane Preparation (5 or 10 μ g/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.05 nM [125 I]-neuromedin U25 and increasing concentrations of unlabeled neuromedin U25, and more than 12- fold signal:background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

Table 1. Signal:background and specific binding values obtained in a competition binding assay with NMU1 membrane prep.

	10 μ g/well	5 μ g/well
Signal:background	15.7	20.5
Specific binding (cpm)	4779	4829

SPECIFICATIONS: 1 unit = 5 μ g membrane preparation
Bmax: 1.06 pmol/mg
K_d: 0.08 nM

Species: Human NMU1 (Accession number NM_006056)

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 MgCl₂, 1 mM CaCl₂, 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 ¹²⁵I-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: Membranes protein were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.
- STORAGE/HANDLING:** Maintain frozen at -70°C for up to 2 years. Do not freeze and thaw.
- REFERENCES:** Brighton PJ *et al.* (2004a) Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol. Rev.* 56: 231-48
- 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.
- 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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Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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