

CHEMISCREEN™ MEMBRANE PREPARATION
RECOMBINANT HUMAN NMU2 NEUROMEDIN U RECEPTOR

CATALOG NUMBER:	HTS098M	QUANTITY:	200 units
LOT NUMBER:		VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: 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 and the CA1 region of the hippocampus, and to 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). Millipore's NMU2 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 0.58 nM for [¹²⁵I]-neuromedin U-25. With 5 µg/well NMU2 Membrane Prep and 0.25 nM [¹²⁵I]-neuromedin U-25, a greater than 10-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

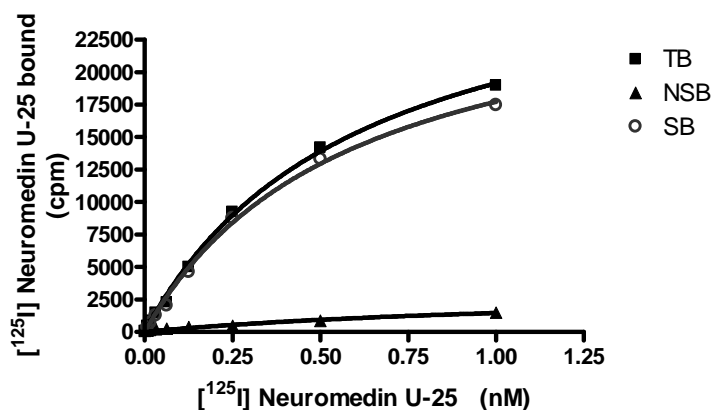


Figure 1. Saturation binding for NMU2. 5 µg/well NMU2 Membrane Preparation was incubated with increasing amount of [¹²⁵I]-neuromedin U-25 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of greater than 500-fold excess unlabeled neuromedin U-25. Specific binding (SB) was determined by subtracting NSB from TB.

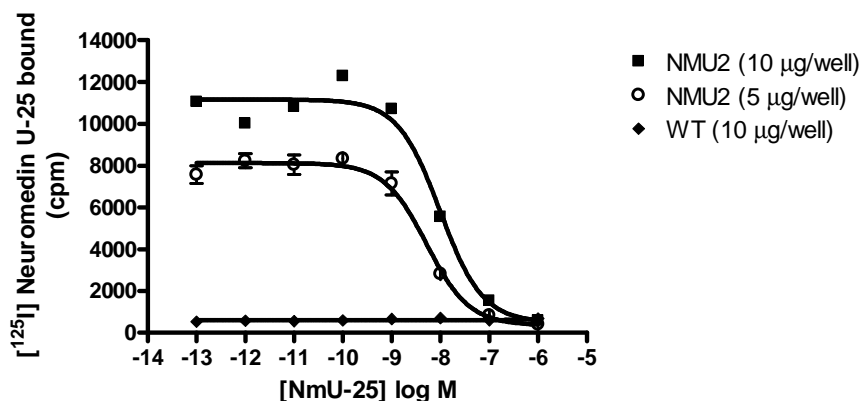


Figure 2. Competition binding for NMU2. NMU2 Membrane Preparation (5 or 10 µg/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 0.25 nM [¹²⁵I]-neuromedin U-25 and increasing concentrations of unlabeled neuromedin U-25, and more than 10-fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with NMU2 membrane prep.

	10µg/well	5µg/well
Signal:background	22.4	22.5
Specific binding (cpm)	10666	7780

SPECIFICATIONS: 1 unit = 5 µg membrane preparation
 Bmax: 3.92 pmol/mg
 K_d: 0.58 nM

Species: Human NMU2 (Accession number NM_006056)

HOST CELLS: Chem-1, an adherent mammalian cell line without any endogenous NMU2 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, an FC 96-well harvest plate (Millipore cat. # MAHF C1H) 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: [¹²⁵I]-neuromedin U-25 (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 10-fold signal:background with ¹²⁵I-labeled neuromedin U-25 at 0.25 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:

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:

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 G $\alpha_{q/11}$ and G α_i and an irreversible ligand-receptor interaction. *Mol. Pharmacol.* 66: 1544-56.

Howard AD *et al.* (2000) Identification of receptors for neuromedin U and its role in feeding. *Nature* 406: 70-74.

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.

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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