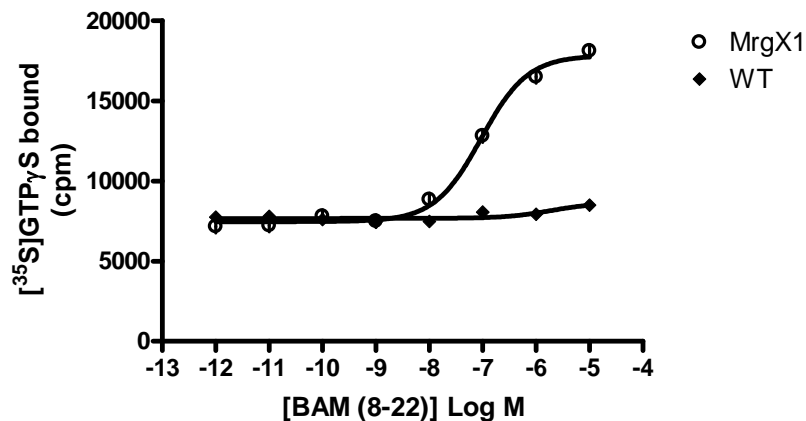


**CATALOG NUMBER:** HTS122M      **QUANTITY:** 200 units  
**LOT NUMBER:**      **VOLUME/CONCENTRATION:** 1 mL, 1 mg/mL

**BACKGROUND:** The Mas-related gene family (Mrg), also known as SNSR (sensory neuron-specific G protein-coupled receptors), are expressed mainly in the dorsal root ganglia and appear to be involved in nociception and other sensory processes. MrgX1/SNSR4 is activated by the neuropeptide BAM22, and it mediates neuronal synaptic transmission and ion channel activity (Lembo *et al.*, 2002; Burstein *et al.*, 2006, Chen and Ikeda, 2004). Millipore's MrgX1 Membrane Preparation for GTP $\gamma$ S Assay is a crude membrane preparation made from the proprietary stable recombinant cell line that is also used for Millipore's MrgX1 Calcium Optimized Cell Line (HTS122C). This MrgX1 Membrane Preparation has been optimized for low basal activity in GTP $\gamma$ S binding assays; thus it is an ideal HTS tool for screening of compounds for agonist and antagonist activities. The membrane preparations exhibits EC<sub>50</sub> of 93.7 nM for BAM(8-22) in a GTP $\gamma$ S binding assay.

**APPLICATIONS:** GTP $\gamma$ S Binding Assay



**Figure 1. Binding of [<sup>35</sup>S]-GTP $\gamma$ S to MrgX1 membrane preparation.** 5  $\mu$ g/well MrgX1 Membrane Preparation (catalog # HTS122M) and Wild-type Chem-1 Membrane Preparation (WT; Millipore HTS000MC1) were incubated with 0.3 nM [<sup>35</sup>S]-GTP $\gamma$ S, 0.5  $\mu$ M GDP and increasing amounts of unlabeled BAM(8-22). Bound radioactivity was determined by filtration and scintillation counting.



## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN MrgX1 RECEPTOR

SPECIFICATIONS: 1 unit = 5 µg  
EC50 in GTP $\gamma$ S binding assay by BAM (8-22): ~ 93.7 nM

Species: Human MrgX1 (Accession number NM\_147199)

HOST CELLS: Chem-1, an adherent cell line with no detectable endogenous MrgX1 expression

GTP $\gamma$ S ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [<sup>35</sup>S]-GTP $\gamma$ S (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM MgCl<sub>2</sub>/0.5 µM GDP in a nonbinding 96-well plate. Unlabeled BAM (8-22) was added to the final concentration indicated in Figure 1 (final volume 100 µL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (Millipore MAHF B1H) previously prewetted with water. The plate is washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then dried and counted.

### PRESENTATION:

One vial contains enough membranes for at least 200 assays (units), where one unit is the amount of membrane that will yield greater than 1000 cpm specific BAM(8-22)-stimulated [<sup>35</sup>S]-GTP $\gamma$ S binding.

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

Packaging method: Membrane protein was 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:

Burstein ES *et al.* (2006) Characterization of the Mas-related gene family: structural and functional conservation of human and rhesus MrgX receptors. *Br. J. Pharmacol.* 147: 73-82.

Chen H and Ikeda SR (2004) Modulation of ion channels and synaptic transmission by a human sensory neuron-specific G-protein-coupled receptor, SNSR4/mrgX1, heterologously expressed in cultured rat neurons. *J. Neurosci.* 24: 5044-5053.

Lembo PMC *et al.* (2002) Proenkephalin A gene products activate a new family of sensory neuron-specific GPCRs. *Nat. Neurosci.* 5: 201-209.

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