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PRODUCT DATASHEET

ChemiScreen™ MrgD Mas-Related Gene Family Membrane Preparation

CATALOG NUMBER:	HTS206M	**QUANTITY:**	200 units
LOT NUMBER:	1976505	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

The Mas-related genes (Mrgs), also known as sensory neuron-specific G-protein coupled receptors (SNSRs), are selectively expressed in subpopulations of sensory neurons involved in the perceptor of pain (Dong *et al*., 2001). The Mrg family in rodents consists of 50 receptors in the MrgA-MrgH subfamilies, but humans contain fewer Mrg members. MrgD (also known as TGR7) is found across species from rodents to humans, and its expression is restricted to nonpeptidergic, small-diameter, IB4+ dorsal root ganglion (DRG) neurons. MrgD binds β-alanine, but not L-alanine, which results in increased intracellular calcium and the suppression of forskolin-induced cAMP production, thus suggesting coupling to Gq and Gi proteins (Shinohara *et al*., 2004). β-alanine-induced activation of MrgD inhibits KCNQ2/3-mediated M-currents in cells overexpressing MrgD and KCNQ2/3 and also in cultured DRG neurons, which indicates that MrgD enhances nociceptive signaling (Crozier *et al*., 2007). MrgD 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 MrgD interactions with its ligands. The cell line exhibits a calcium response with EC50s of 11 µM for β-alanine. The membrane preparations exhibit EC50s of 69 µM for β-alanine in a GTPγS binding assay.

APPLICATIONS:

GTPγS Binding and Radioligand Binding Assay

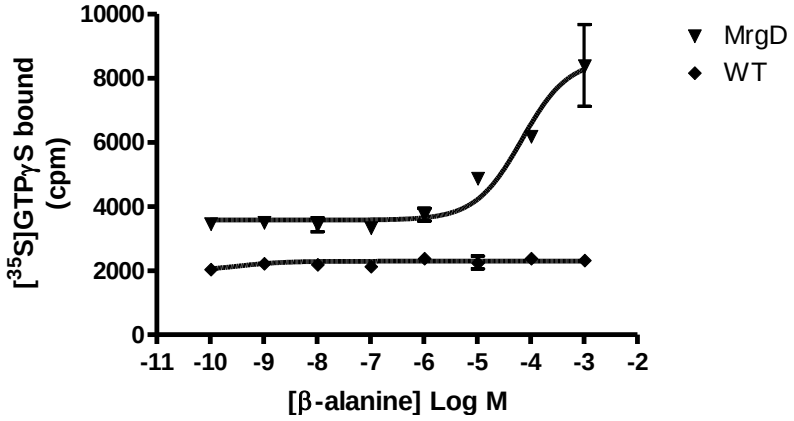

Figure 1. Binding of [^{35}S]-GTPγS to MrgD Membrane Preparation. 5 µg/well of MrgD Membrane Preparation (catalog # HTS206M) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled β-alanine. Bound radioactivity was determined by filtration and scintillation counting.

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SPECIFICATIONS: 1 unit = 5 µg
EC50 in GTPγS binding assay by β-alanine: ~ 69 µM

Species: Full-length human MRGPRD cDNA encoding MrgD (Accession Number: NM_198923).

HOST CELLS: Chem-1, an adherent rodent cell line lacking endogenous MrgD.

RECOMMENDED ASSAY CONDITIONS: Membranes are permeabilized by addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4/100 mM NaCl/10 mM $MgCl_2$/0.5 µM GDP in a nonbinding 96-well plate. Unlabeled β-alanine 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 a GF/B filter plate (EMD 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.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 1000 cpm specific β-alanine -stimulated [^{35}S]-GTPγS binding.

The MrgD membrane preparation is expected to be functional in a radioligand binding assay; however, the end user will need to determine the optimal radiolabeled ligand for use with this product.

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^{o}C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Crozier RA *et al.* (2007). MrgD Activation Inhibits KCNQ/M-Currents and Contributes to Enhanced Neuronal Excitability. *J. Neurosci.* 27(16): 4492-4496.

2. Dong X et al. (2001). A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell* 106: 619-632.

3. Shinohara T *et al.* (2004). Identification of a G protein-coupled receptor specifically responsive to β-alanine. *J. Biol. Chem.* 279: 23559-23564.