

PRODUCT DATASHEET
ChemiScreen™ Motilin Receptor Membrane Preparation

CATALOG NUMBER:	HTS121M	QUANTITY:	200 units
LOT NUMBER:	SC858024	VOLUME/CONCENTRATION:	1 mL, 1 mg/mL

BACKGROUND: Motilin is a 22 amino acid peptide that potently stimulates gastrointestinal contractility. The biological effects of motilin are mediated by a G_q-coupled seven transmembrane protein, currently termed motilin receptor (MR) that shares significant sequence similarity with the ghrelin receptor (Feighner *et al.*, 1999). The motilin receptor is also activated by the antibiotic erythromycin. This interaction appears to mediate some of the gastrointestinal side effects of erythromycin. Although motilides (non-antibiotic derivatives of erythromycin) such as ABT-229 have been investigated for treatment of diabetic gastroparesis, the effectiveness has been limited by tachyphylaxis (decreased response to ligand) resulting from receptor downregulation (Thielemans *et al.*, 2005). Agonists of the motilin receptor with reduced desensitization activity remain a potential treatment for disorders of gastric motility. The motilin 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 motilin receptor interactions with motilin. The membrane preparations exhibit a K_d of 0.38 nM for [¹²⁵I]-Motilin. With 5.0 μg/well of Motilin Membrane Prep and 0.5 nM [¹²⁵I]-Motilin, a greater than 30-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand Binding Assay

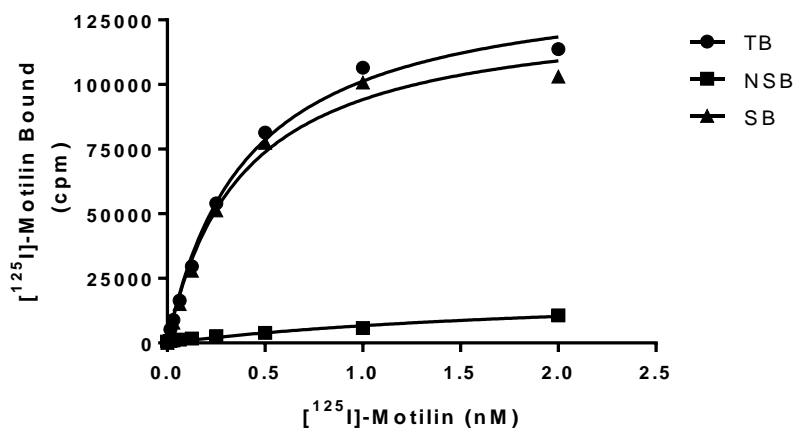


Figure 1. Saturation Binding for Motilin Receptor. 5.0 μg/well Motilin Membrane Preparation was incubated with increasing amounts of [¹²⁵I]-Motilin in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 200-fold excess unlabeled motilin. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample from lot SC858024.

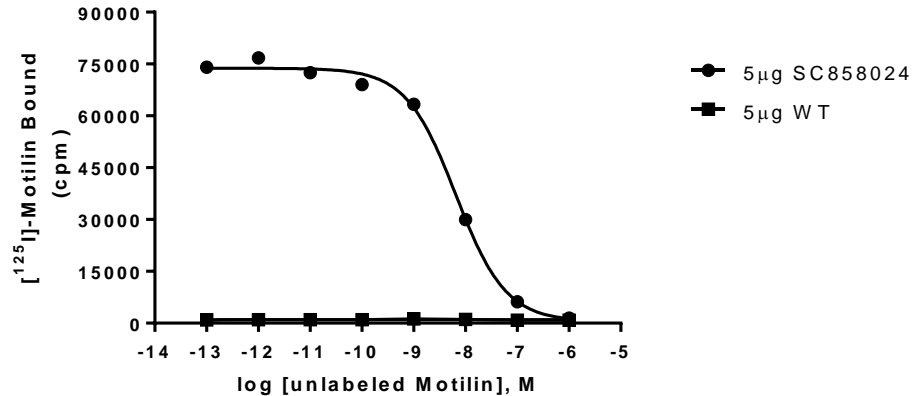


Figure 2. Competition Binding for Motilin Receptor. Motilin Receptor Membrane Preparation (5 μg/well) or Wild-Type Chem-1 membrane preparation (WT; Catalog # HTS000MC1) was incubated with 0.5 nM [¹²⁵I]-Motilin and increasing concentrations of unlabeled motilin, and more than a 30-fold signal:background was obtained. The data are from a representative sample from lot SC858024.

SPECIFICATIONS: 1 unit = 5 μg
 B_{max} for [¹²⁵I]-Motilin binding: 10.6 pmol/mg protein
 K_d for [¹²⁵I]-Motilin binding: 0.38 nM
 Signal:background: >30-fold

TRANSFECTION: Full-length human cDNA encoding the Motilin Receptor (Accession Number: NM_001507).

HOST CELLS: Chem-1, an adherent mammalian cell line with no endogenous Motilin Receptor 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 2 h at room temperature. Prior to filtration, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50 mM HEPES, pH 7.4, 0.5% BSA. The binding reactions are transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The wells are then 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]-Motilin (PerkinElmer # NEX378).

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

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than a 30-fold signal:background ratio with [¹²⁵I]-Motilin 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 1.0 mg/mL 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. Avoid repeated freeze/thaw cycles.

REFERENCES:

1. Feighner SD *et al.* (1999). Receptor for motilin identified in the human gastrointestinal system. *Science* 284:2184-2188.
2. Thielemans L *et al.* (2005). Desensitization of the human motilin receptor by motilides. *J. Pharmacol. Exp. Ther.* 313:1397-1405.

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