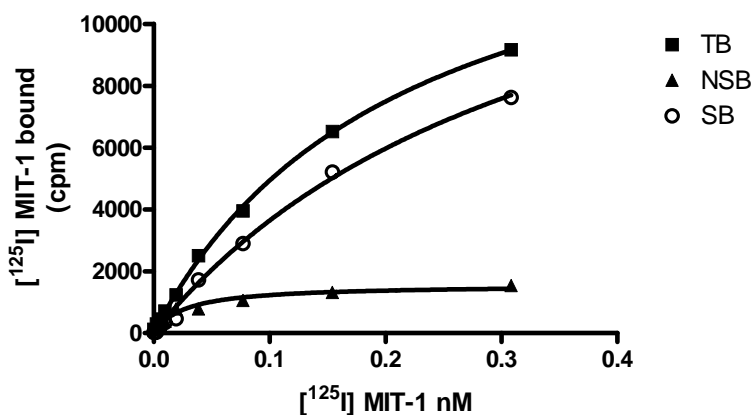


## CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN PK<sub>2</sub> RECEPTOR

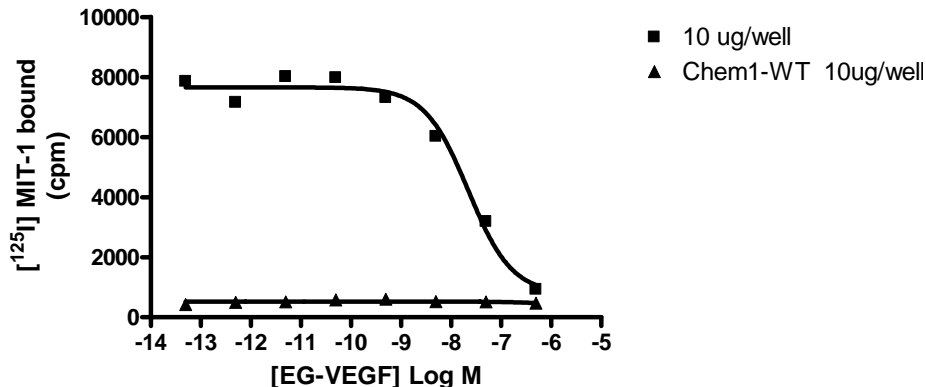
<b>CATALOG NUMBER:</b>	HTS182M	<b>QUANTITY:</b>	200 units
<b>LOT NUMBER:</b>		<b>VOLUME/CONCENTRATION PER VIAL:</b>	2 mL, 1 mg/mL

**BACKGROUND:** Prokineticins, also known as endocrine gland vascular endothelial growth factors (EG-VEGF), are two ~10 kD secreted proteins originally described to mediate angiogenesis and gastrointestinal smooth muscle contraction (Li *et al.*, 2001; LeCouter *et al.*, 2003). Subsequently, prokineticins have been found to mediate central nervous system functions including circadian rhythms and olfactory bulb development (Cheng *et al.*, 2002; Ng *et al.*, 2005). Two G<sub>q</sub>-coupled receptors, PK<sub>1</sub> and PK<sub>2</sub> (also known as GPR73a and GPR73b), mediate cellular responses to prokineticins (Lin *et al.*, 2002). Millipore's PK<sub>2</sub> 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 agonists and antagonists at PK<sub>2</sub>. The membrane preparations exhibit a K<sub>d</sub> of 0.36 nM for [<sup>125</sup>I]-MIT-1. With 0.3 nM [<sup>125</sup>I]-MIT-1, 10 μg/well PK<sub>2</sub> Membrane Prep typically yields greater than 5-fold signal-to-background ratio.

**APPLICATIONS:** Radioligand binding assay and GTPγS binding.



**Figure 1. Saturation binding for PK<sub>2</sub>.** 10 μg/well PK<sub>2</sub> Membrane Preparation was incubated with increasing amount of <sup>125</sup>I-labeled MIT-1 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled EG-VEGF. Specific binding (SB) was determined by subtracting NSB from TB.



**Figure 2. Competition binding for PK<sub>2</sub>.** 10  $\mu$ g/well PK<sub>2</sub> Membrane Preparation and wild-type Chem-1 Membrane Preparation (Millipore catalog # HTS000MC1) were incubated in a 96-well plate with 0.3 nM [<sup>125</sup>I]-labeled MIT-1 and increasing concentrations of unlabeled EG-VEGF. More than 5-fold signal:background was obtained with unlabeled EG-VEGF.

**Table 1.** Signal:background and specific binding values obtained in a competition binding assay with PK<sub>2</sub> membrane prep and unlabeled EG-VEGF.

	10 $\mu$ g/well
Signal:background	9.80
Specific binding (cpm)	6874

SPECIFICATIONS: 1 unit = 10  $\mu$ g

$B_{max}$  for [<sup>125</sup>I]- MIT-1 binding: 0.52 pmol/mg protein

$K_d$  for [<sup>125</sup>I]- MIT-1 binding: ~0.36 nM

TRANSFECTION: Full-length human GPR73L1 cDNA encoding PK<sub>2</sub> (Accession Number: NM\_144773)

HOST CELLS: Chem-1, an adherent mammalian cell line with minimum amount of endogenous PK<sub>2</sub> 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 MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.2% BSA, filtered and stored at 4°C

Radioligand: [<sup>125</sup>I] MIT-1 (Perkin Elmer#: NEX-410)

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

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One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 5-fold signal:background with <sup>125</sup>I-labeled MIT-1 at 0.3 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 1 mg/mL in packaging buffer, and dispensed at 1 mL/vial. Vials were 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:**

Cheng MY *et al.* (2002) Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417: 405-10.

LeCouter J *et al.* (2003) Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. *Nat. Med.* 8: 913-7.

Li M *et al.* (2001) Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle. *Mol. Pharmacol.* 59: 692-8.

Lin DC *et al.* (2002) Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. *J. Biol. Chem.* 277: 19276-80.

Ng KL *et al.* (2005) Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science* 308: 1923-7.

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