eurofins | Discovery Services

PRODUCT DATASHEET

ChemiScreen™ PK1 Prokineticin Receptor Membrane Preparation

CATALOG NUMBER:	HTS074M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	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 Gq-coupled receptors, PK1 and PK2 (also known as GPR73a and GPR73b), mediate cellular responses to prokineticins (Lin *et al.,* 2002). PK1 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 PK1 interactions and its ligands. The membrane preparations exhibit a Kd of 0.11 nM for [^{125}I]-Mamba Intestinal Toxin-1 (MIT-1). With 0.2 nM [^{125}I]-MIT-1, 2.5 μg/well and 5 μg/well PK1 Membrane Prep typically yield greater than 2-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

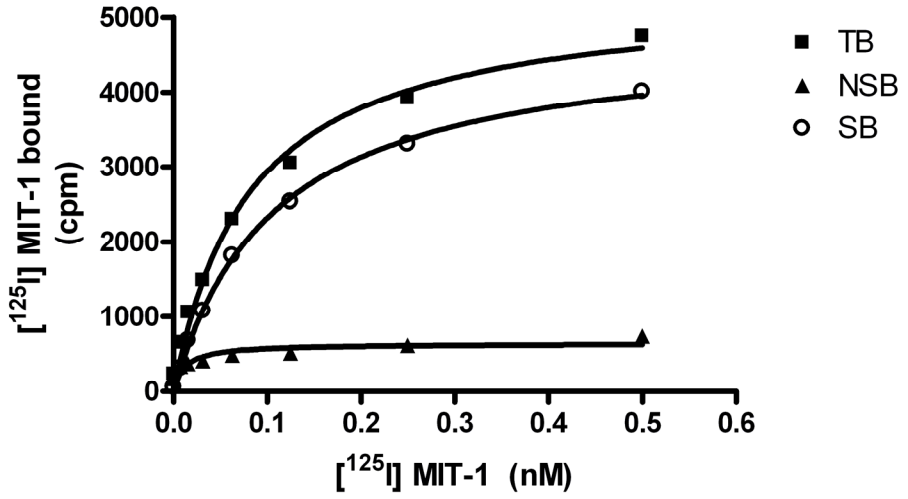

Figure 1. Saturation binding for PK1. 5 μg/well PK1 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled MIT-1 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled PK1. Specific binding (SB) was determined by subtracting NSB from TB. Sample data

Eurofins Pharma Bioanalytics Services US Inc.

15 Research Park Drive
St Charles MO 63304
USA

T +1 844 522 7787
F +1 636 362 7131
www.eurofins.com

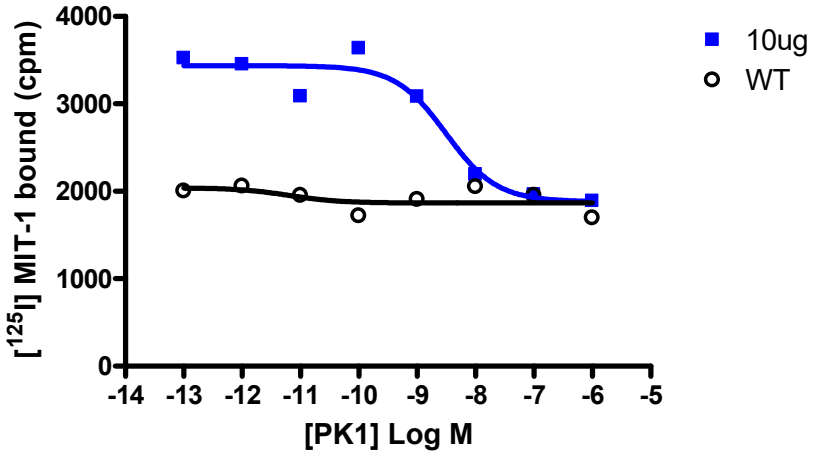

Figure 2. Competition binding for PK1. PK1 Membrane Preparation (10 μg/well) and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.2 nM ^{125}I-labeled MIT-1 and increasing concentrations of unlabeled PK1, and subjected to filtration binding. Sample data.

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
Bmax: for [^{125}I] MIT-1 binding: 0.58 pmol/mg protein;
K_d: [^{125}I] MIT-1 binding: ~ 0.11 nM
Signal:Background Ratio: 2-fold

TRANSFECTION: Full-length human GPR73 cDNA, encoding PK1 (Accession Number: NM_138964)

Species: Human

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous PK1 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, a GF/C 96-well filter plate 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 $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{125}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.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 2-fold signal:background with ^{125}I-labeled MIT-1 at 0.2 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 the indicated concentration 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. 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.* (2002) 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.