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PRODUCT DATASHEET

ChemiScreen™ PK_2 Prokineticins Membrane Preparation

CATALOG NUMBER:	HTS182M	**QUANTITY:**	200 units
LOT NUMBER:	JH1917577	**VOLUME/CONCENTRATION:**	1 mL, 2 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_q-coupled receptors, PK_1 and PK_2 (also known as GPR73a and GPR73b), mediate cellular responses to prokineticins (Lin *et al*., 2002). PK_2 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_2. The membrane preparations exhibit a K_d of 0.36 nM for [^{125}I]-MIT-1. With 0.3 nM [^{125}I]-MIT-1, 10μg/well PK_2 Membrane Prep typically yields a 2-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay

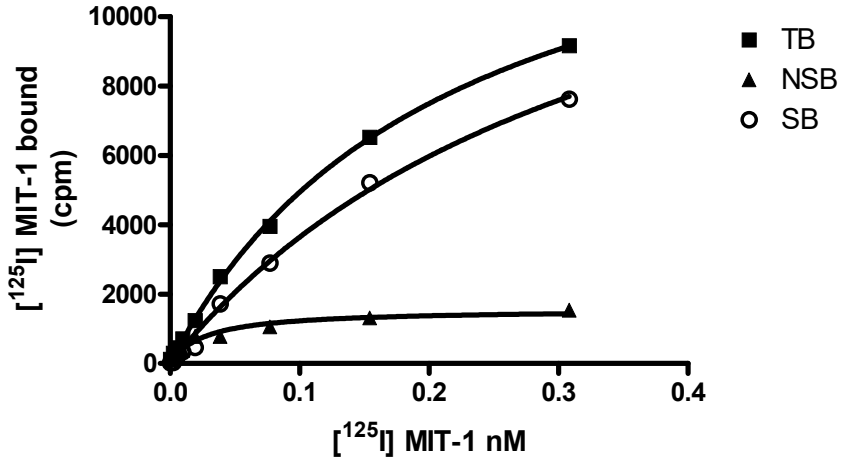

Figure 1. Saturation binding for PK_2. 10 μg/well PK_2 Membrane Preparation was incubated with increasing amount of ^{125}I-labeled MIT-1 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled EG-VEGF. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

Eurofins Pharma Bioanalytics Services US Inc.

6 Research Park Drive
St Charles MO 63304
USA

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

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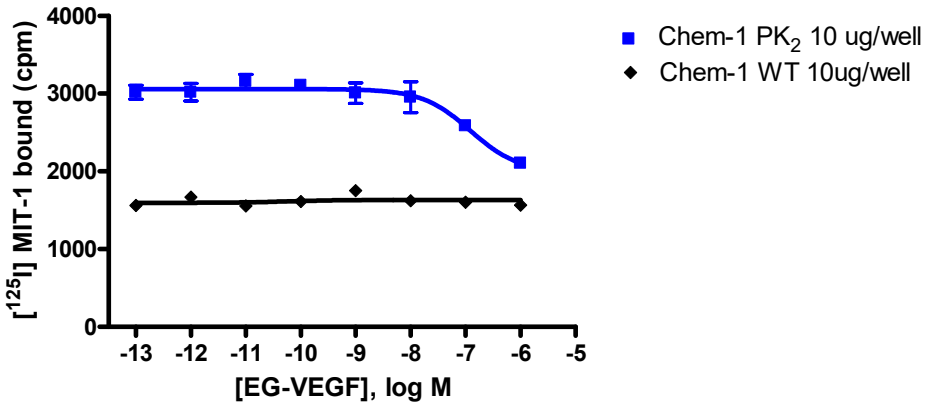

Figure 2. Competition binding for PK_2. 10 µg/well PK_2 Membrane Preparation and wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated in a 96-well plate with 0.3 nM ^{125}I-labeled MIT-1 and increasing concentrations of unlabeled EG-VEGF. A 2-fold signal:background was obtained with unlabeled EG-VEGF. Representative sample data.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{125}I]- MIT-1 binding: 0.52 pmol/mg protein
K_d for [^{125}I]- MIT-1 binding: ~0.36 nM
Signal:background: >2-fold

TRANSFECTION: Full-length human GPR73L1 cDNA encoding PK_2 (Accession Number: NM_144773)

HOST CELLS: Chem-1, an adherent mammalian cell line with minimum amount of endogenous PK_2 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 (EMD 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 $MgCl_2$, 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 a 2-fold signal:background with ^{125}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: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80ºC.

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:

1. Cheng MY *et al.* (2002) Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417: 405-10.
2. LeCouter J *et al.* (2003) Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. *Nat. Med.* 8: 913-7.
3. Li M *et al.* (2001) Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle. *Mol. Pharmacol.* 59: 692-8.
4. 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.
5. Ng KL *et al.* (2005) Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science* 308: 1923-7.