

CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN KAPPA OPIOID RECEPTOR

CATALOG NUMBER: HTS095M **QUANTITY:** 200 units
LOT NUMBER: **VOLUME/CONCENTRATION:** 1 mL, 2 mg/mL

BACKGROUND: Opiates derived from the opium poppy, *Papaver somniferum*, have been used in for millenia for their anti-diarrheal, analgesic and euphoric properties. More recently, endogenous peptides, enkephalins, dynorphins, and endorphins, were found to bind to the same sites as opiate alkaloids. The receptors for the classical opioids are three related GPCRs, μ , κ , and δ , that activate Gi/o to reduce intracellular cAMP levels. Most clinically used opioids function by activation of the μ opioid receptor. Activation of the κ opioid receptor by selective agonists also produces analgesia, primarily mediated by spinal sites, but causes dysphoria and psychosis instead of euphoria. The κ receptor at central and peripheral sites is also largely responsible for the anti-diarrheal effects of opiates (Dhawan *et al.*, 1996). Millipore's 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 κ interactions with diprenorphine. The membrane preparations exhibit a Kd of 2.3-3.7 nM for [³H]-diprenorphine. With 10 μ g/well κ Membrane Prep and 2.5 nM [³H]-diprenorphine, a greater than 3-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

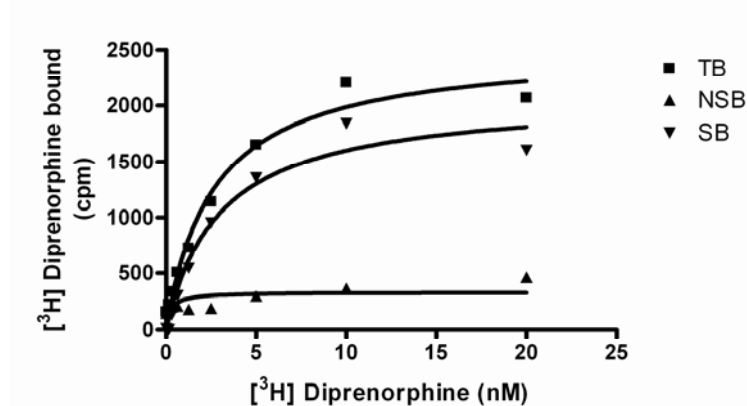


Figure 1. Saturation binding for κ . 10 μ g/well κ Membrane Preparation was incubated with increasing amount of [³H]-diprenorphine in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled U-69,593. Specific binding (SB) was determined by subtracting NSB from TB.

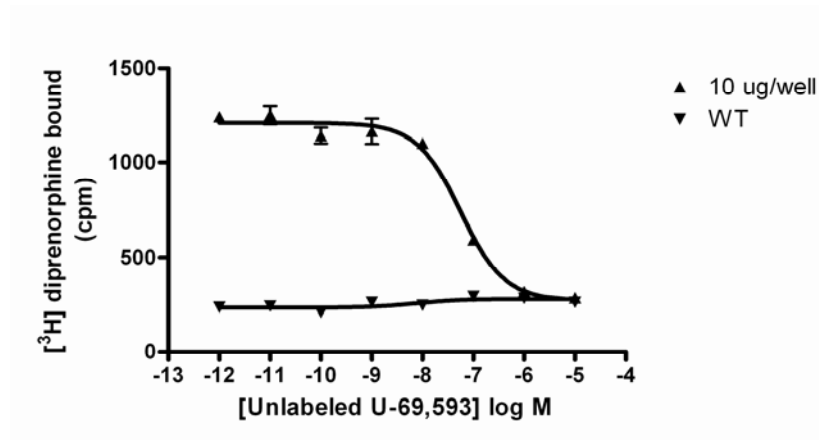


Figure 2. Competition binding for κ . κ Membrane Preparation (10 μ g/well) or Wild-Type Chem-1 membrane preparation (WT; Chemicon Catalog # HTS000MC1) was incubated with 2.5 nM [3 H]-diprenorphine and increasing concentrations of unlabeled U-69,593, and more than 3- fold signal:background was obtained.

Table 1. Signal:background and specific binding values obtained in a competition binding assay with κ membrane prep.

	10 μ g/well
Signal:background	4.47
Specific binding (cpm)	939

SPECIFICATIONS: 1 unit = 10 μ g membrane preparation
 Bmax: 21.73 pmol/mg
 K_d: 3.0 nM

Species: Human full length OPRK1 cDNA encoding κ (Accession number NM_000912)

HOST CELLS: Chem-1, an adherent mammalian cell line with no detectable endogenous κ 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 Binding Buffer. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Binding Buffer. The plate is dried and counted.

Binding buffer: 50 mM Tris-HCl pH 7.4, filtered and stored at 4°C

Radioligand: [3 H] diprenorphine (Perkin Elmer # NET1121)



One package contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield greater than 3-fold signal:background with ^3H -labeled diprenorphine at 2.5 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 the indicated concentration in 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:

Dhawan BN *et al.* (1996) International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* 48: 567-92:

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC
PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

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