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PRODUCT DATASHEET

ChemiScreen™ Kappa Opioid Family Membrane Preparation

CATALOG NUMBER:	HTS095M	**QUANTITY:**	200 units
LOT NUMBER:	SC20170427	**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). κ Membrane preparations are crude membrane preparations made from our proprietary stable recombinant cell lines to ensure high-levels 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 2.7nM for [^{3}H]-U69,593. With 10 μg/well of κ Membrane Prep and 5 nM [^{3}H]-U69,953, a 2-fold signal-to-background ratio was obtained.

APPLICATIONS: Radioligand binding assay

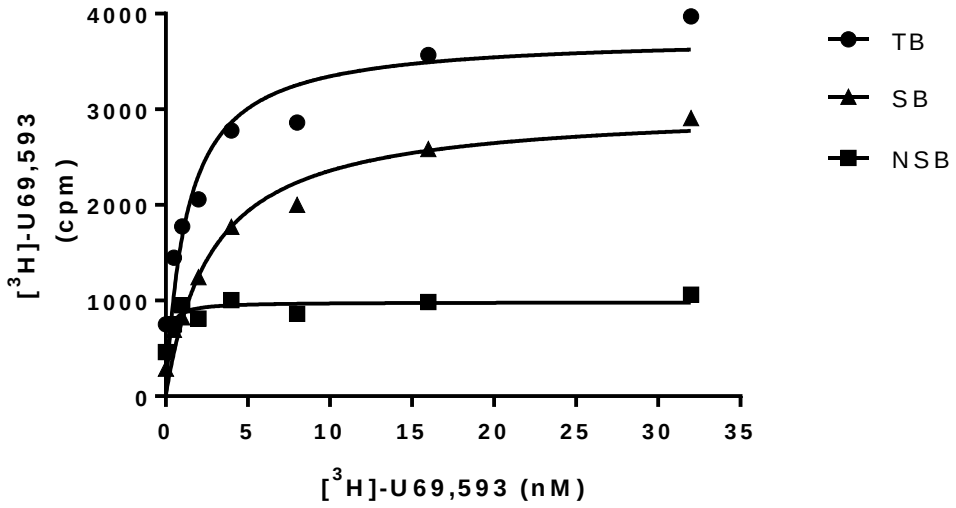

Figure 1. Saturation Binding for κ. 10 μg/well of κ Membrane Preparation were incubated with increasing amounts of [^{3}H]- U69,953 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 500-fold excess unlabeled U-69,593. Specific binding (SB) was determined by subtracting NSB from TB. The data are from a representative sample of lot SC20170427.

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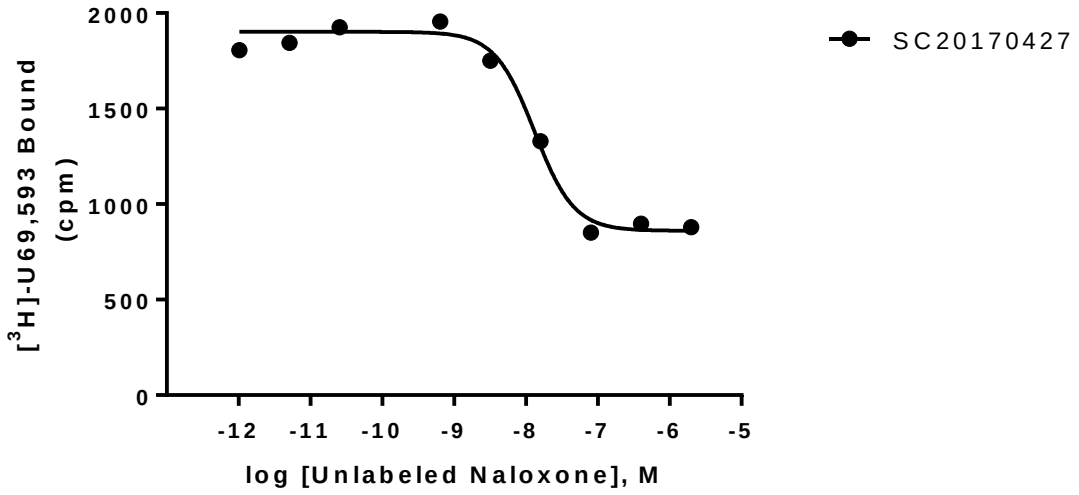

Figure 2. Competition Binding for κ. 10 μg/well of κ Membrane Preparation membrane preparation was incubated with 5 nM [^{3}H]-U69,593 and increasing concentrations of unlabeled Naloxone, and more a 2-fold signal:background ratio was obtained. The data are from a representative sample of lot SC20170427.

SPECIFICATIONS: 1 unit = 10 μg membrane preparation
Bmax: for [^{3}H]-U69,593 Binding: 4.5 pmol/mg protein
K_d: [^{3}H]- U69,593 Binding: 2.79 nM
Signal:Background Ratio: 2-fold

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

Species: Human

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 (cat. # MAHF C1H) is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM Tris buffer, pH 7.4. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with 50mM Tris buffer, pH 7.4. The plate is dried and counted.

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

Radioligand: [^{3}H]- U69,593 (PerkinElmer#: NET952)

Wash Buffer: 50 mM Tris-HCl, pH 7.4.

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 [^{3}H]- U69,593 at 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 the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80^{o}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: