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PRODUCT DATASHEET

ChemiScreen™ CB_1 Cannabinoid Membrane Preparation

CATALOG NUMBER:	HTS019M	**QUANTITY:**	200 units
LOT NUMBER:	22C1504	**VOLUME/CONCENTRATION**	1 mL, 2 mg/mL

BACKGROUND:

CB_1 is a GPCR that is expressed primarily in brain and nervous tissue, and mediates numerous CNS responses such as analgesia, appetite, cognition, memory and locomotor activity. A number of cannabinoid ligands bind to CB_1 and activate $G_{i/o}$-mediated downstream responses, including inhibition of cAMP production and activation of ion channels and MAP kinases. Ligands for CB_1 include exogenous agonists such as Δ^9-THC, the main psychoactive component of the plant *Cannabis sativa*, and endogenous eicosanoid agonists such as anandamide. A number of synthetic agonists such as CP55940 and *R*-(+)-WIN55212, and antagonists, such as SR141716A, for CB_1 have been developed (Howlett *et al*., 2002). CB_1 agonists have clinical utility in analgesia and antiemetic properties, whereas CB_1 antagonists show promise for treatment of appetite in obesity disorders. The CB_1 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 for agonists and antagonists of CB1. The membrane preparations exhibit a K_d of 2.15 nM for [^{3}H]-CP55940. In the presence of 2 nM [^{3}H]-CP55940 with WIN55212 as the unlabeled competitor, 10 µg/well of CB_1 Membrane Prep typically yields ≥2.5-fold signal-to-background ratio.

APPLICATIONS:

Radioligand Binding Assay

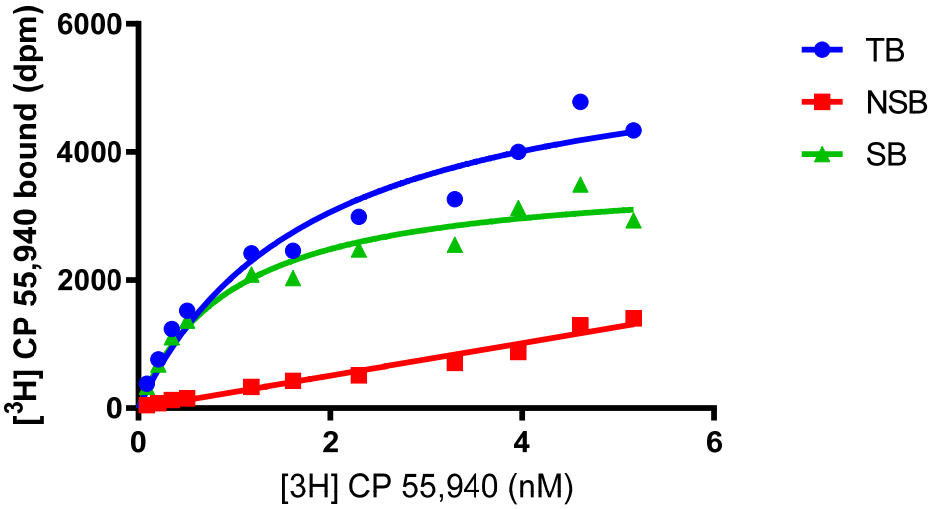

Figure 1. Saturation Binding for CB_1. 7.5 µg/well of CB_1 Membrane Preparation was incubated with increasing amounts of [^{3}H]-CP55940 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 5000-fold excess unlabeled WIN55212. Specific binding (SB) was determined by subtracting NSB from TB.

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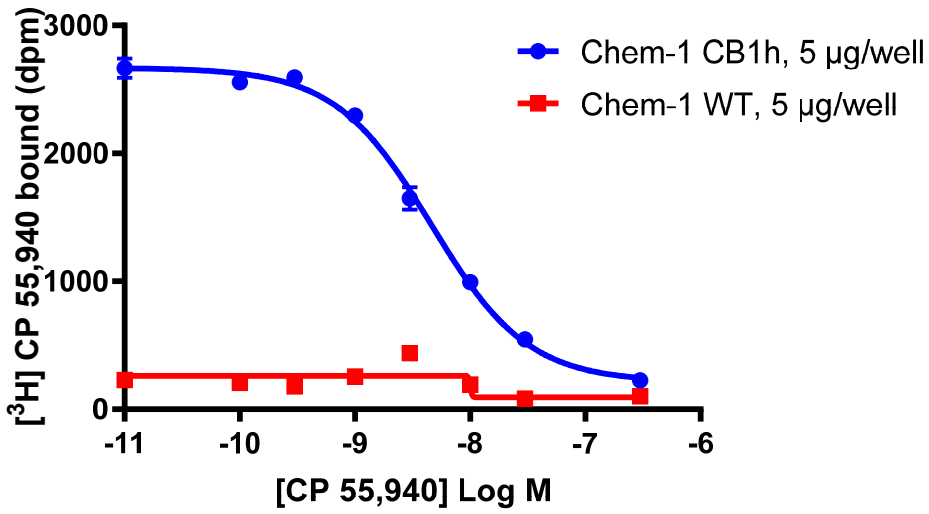

Figure 2. Competition Binding for CB_1. 7.5 µg/well of CB_1 Membrane was incubated with 2 nM [^{3}H]-CP55940 and increasing concentrations of unlabeled CP55940 and were subjected to filtration binding. Greater than 1.5-fold signal:background ratio was obtained.

SPECIFICATIONS: 1 unit = 10 µg
B_{max} for [^{3}H]-CP55940 Binding: 3.055 pmol/mg protein
K_d for [^{3}H]-CP55940 Binding: 0.964 nM
Signal:Background: ≥2.5-fold

HOST CELLS: Chem-1, an adherent mammalian cell line without detectable endogenous CB_1 expression.

Species: Human CB_1 (Accession number X54937)

RECOMMENDED BINDING ASSAY CONDITIONS: Membranes were mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a non-binding 96-well plate and incubated for 30 min at room temperature. Prior to filtration, an FC 96-well harvest plate was coated with 0.3% polyethyleneimine. The binding reactions were transferred to the filter plate, and washed 7 times (1 mL per well per wash) with Wash Buffer. The wells were then dried and counted to determine receptor-associated radioligand binding.

Binding Buffer: 50 mM Tris/HCl, pH 7.4, 2.5 mM EDTA/Tris, 5 mM $MgCl_2$, 1 µg/ml Leupeptin, 1 µM Pepstatin, 10 µg/ml trypsin inhibitor, 0.3% BSA, stored at 4°C. Ligands were diluted in binding buffer containing 10% DMSO, and were then added to membranes such that the final DMSO concentration was 1%.

Radioligand: [^{3}H]-CP55940 (PerkinElmer#: NET1051)

Wash Buffer: 50 mM Tris/HCl, pH 7.4, 0.5% BSA, stored at 4°C.

PRESENTATION:

One vial contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield approximately a 1.5-fold signal:background ratio with [^{3}H]-CP55940 at 2 nM.

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 2 mg/mL in packaging buffer, dispensed at 1 mL per vial, 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 this product.

REFERENCES:

1. Howlett AC *et al.* (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* 54:161-202.

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NOT FOR HUMAN OR ANIMAL CONSUMPTION.