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PRODUCT DATASHEET

ChemiScreen™ CB_2 Cannabinoid Membrane Preparation

CATALOG NUMBER:	HTS020M	**QUANTITY:**	200 units
LOT NUMBER:	22G2708	**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND: Cannabinoid compounds include exogenous drugs such as Δ^9-THC, the main psychoactive component of the plant *Cannabis sativa*, and endogenous mediators, such as anandamide, that belong to eicosanoid family. The biological effects of cannabinoids are mediated by a family of two G_i-coupled 7-transmembrane receptors, CB_1 and CB_2. The CB_1 receptor is found primarily in brain and mediates the psychoactive effects of cannabinoid ligands. The CB_2 receptor is expressed mainly in immune cells, including mast cells and CD40-activated B cells, where it mediates proliferation and inhibition of migration (Howlett *et al.*, 2002). Activation of CB_2 inhibits the development of liver fibrosis (Julien *et al.*, 2005). In bone, CB_2 is expressed in both osteoblasts and osteoclasts, and functions to prevent bone loss (Ofek *et al.*, 2006). In addition, activation of CB_2 has an antinociceptive effect in animal models of neuropathic, inflammatory, and acute pain; this effect is mediated by release of endogenous opioids in the periphery (Ibrahim *et al.*, 2005). CB_2 membrane preparations are crude membrane preparations made from stable recombinant cell lines with a high-level of GPCR surface expression; thus, they are ideal HTS tools for screening for agonists and antagonists of CB_2.

APPLICATIONS: Radioligand binding assay and GTPγS binding assay

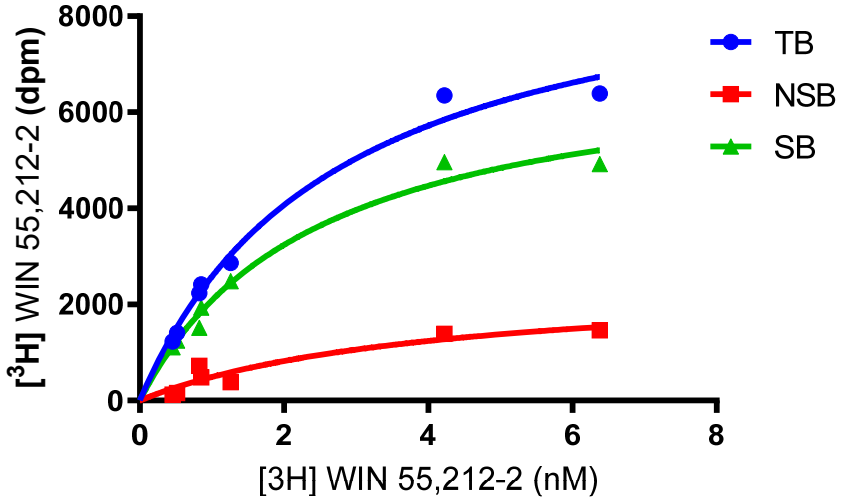

Figure 1. Saturation binding for CB_2. 5 µg/well CB_2 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled WIN 55,212-2 in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled WIN 55,212-2. Specific binding (SB) was determined by subtracting NSB from TB. Sample data from a representative lot.

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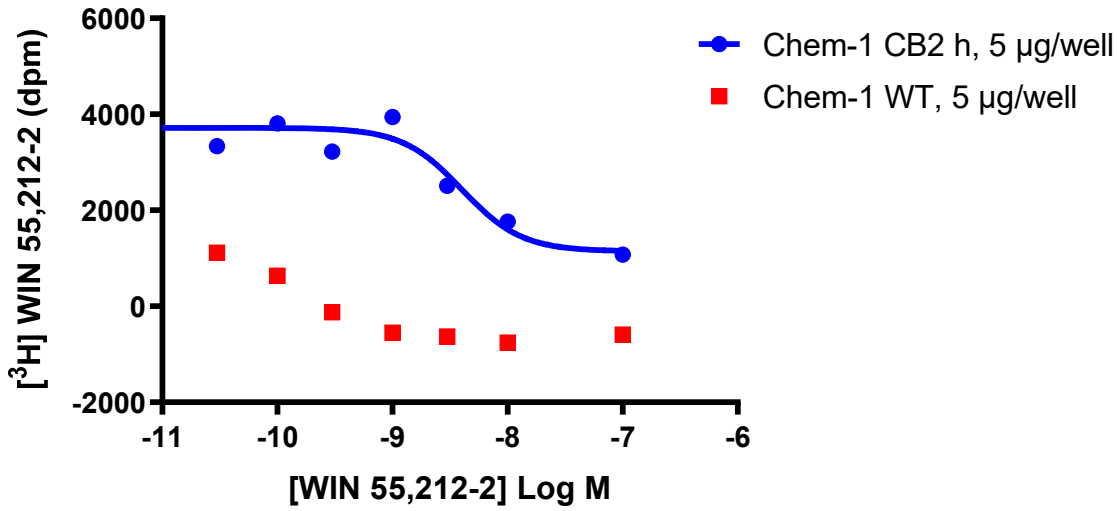

Figure 2. Competition binding for CB_2. CB_2 Membrane Preparation (5 µg/well) or Chem-1 wild-type Membrane Preparation (cat. # HTS000MC1) was incubated with 2.5 nM ^{3}H-labeled WIN 55,212-2 and increasing concentrations of unlabeled WIN 55,212-2. More than 4-fold signal:background was obtained with CB_2 Membrane Preparation at 5 µg/well. Representative sample data.

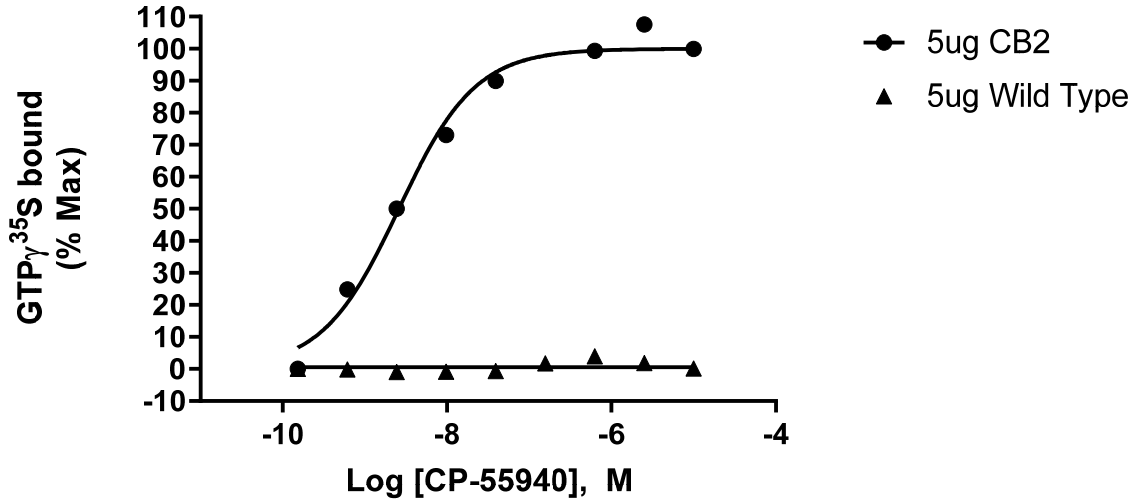

Figure 3. Binding of [^{35}S]-GTPγS to CB_2 membrane preparation. 5 µg/well CB_2 Membrane Preparation (catalog # HTS020M2) and Wild-type Chem-1 Membrane Preparation (catalog # HTS000MC1) were incubated with 0.3 nM [^{35}S]-GTPγS, 10 µM GDP, and increasing amounts of unlabeled CP-55940. Bound radioactivity was determined by filtration and scintillation counting. The data are from a representative lot.

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SPECIFICATIONS: Radioligand Binding:

1 unit = 5 µg
B_{max}: 13.99 pmol/mg
K_d: 2.4 nM
Signal:background: >9-fold

GTPγS Assay:

1 unit = 5 µg
EC50: 2.5 pmol/mg

Species: Human CB_2 (Accession number X74328)

HOST CELLS: Chem-4, an adherent cell line expressing the promiscuous G-proteins.

RECOMMENDED RADIO LIGAND BINDING 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 50 mM 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/Tris (pH 7.4), 5 mM MgCl2, 2.5 mM EGTA and 0.1% BSA, filtered and stored at 4°C

Radioligand: [^{3}H]- WIN 55,212-2 (Perkin Elmer #NET1058)

Wash Buffer: 50 mM Tris-HCl, 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 9-fold signal:background with ^{3}H-labeled WIN 55,212-2 at 0.8 nM.

RECOMMENDED GTPγS ASSAY CONDITIONS: Membranes were permeabilized by the addition of saponin to an equal concentration by mass, then mixed with [^{35}S]-GTPγS (final concentration of 0.3 nM) in 20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 10 µM GDP in a non-binding 96-well plate. Unlabeled CP-55940 was added to the final concentration indicated in Figure 1 (final volume of 100 µL), and incubated for 30 min at 30°C. The binding reactions were transferred to an FB filter plate, previously wetted with water. The wells were washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then dried and counted.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 4-fold signal:background with ^{3}H-labeled CP55,940 at 2.5 nM; or contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 1000 cpm specific CP-55940-stimulated [^{35}S]-GTPγS binding.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA no preservatives. Packaging method: Membranes proteins were adjusted to the indicated concentration in packaging buffer, rapidly frozen, and stored at -80°C.

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

2. Ibrahim MM *et al.* (2005) CB_2 cannabinoid receptor activation produces antinociception by stimulating peripheral release of endogenous opioids. *Proc. Natl. Acad. Sci. USA* 102: 3093-8.

3. Julien B *et al.* (2005) Antifibrogenic role of the cannabinoid receptor CB_2 in the liver. *Gastroenterology* 128: 742-755.

4. Ofek O *et al.* (2006) Peripheral cannabinoid receptor, CB_2, regulates bone mass. *Proc. Natl. Acad. Sci. USA* 103: 696-701.

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