CHEMISCREEN™ MEMBRANE PREPARATION RECOMBINANT HUMAN CB_1 CANNABINOID RECEPTOR

CATALOG NUMBER: HTS019M **QUANTITY:** 200 units

LOT NUMBER: **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. Millipore's 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 at CB1. The membrane preparations exhibit a K_d of 7 nM for [^{3}H]-SR141716A. In the presence of 2 nM [^{3}H]-SR141716A with CP55940 as unlabeled competitor, 10 μg/well CB_1 Membrane Prep yields 4-fold signal-to-background ratio.

APPLICATIONS:

Radioligand binding assay and GTPγS binding.

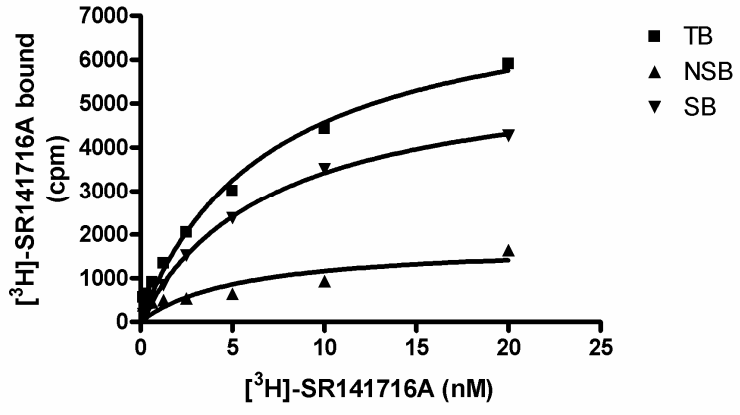

Figure 1. Saturation binding for CB_1. 10 μg/well CB_1 Membrane Preparation was incubated with increasing amount of ^{3}H-labeled SR141716A in the absence (total binding, TB) or presence (nonspecific binding, NSB) of 1000-fold excess unlabeled CP55940. Specific binding (SB) was determined by subtracting NSB from TB.

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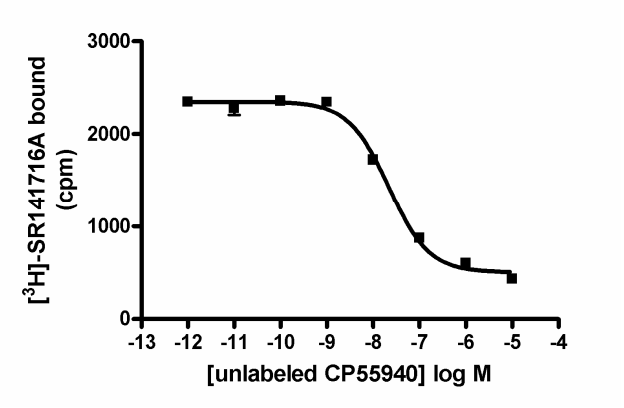

Figure 2. Competition binding for CB_1. 10 µg/well CB_1 Membrane Preparation (HTS019M) was incubated with 2 nM ^{3}H-labeled SR141716A and increasing concentrations of unlabeled CP55,940, and over 4- fold signal:background was obtained.

Table 1. Signal:background and specific binding obtained with CB_1 membrane preparation

	10 µg
Signal:background	4.7
Specific binding (cpm)	1847

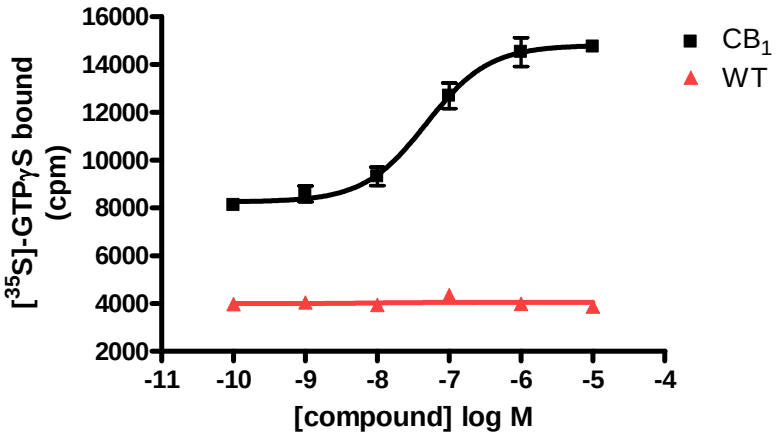

Figure 3. GTPγS binding assay for CB_1. CB_1 Membrane Preparation (HTS019M) at 0.5 units (5 µg) per well and Wild-Type Chem-1 Membrane Preparation (5 µg per well, Millipore cat. # HTS000MC1) was incubated with 0.3 nM [^{35}S]-GTPγS and increasing amounts of unlabeled CP55,940 as described in "[^{35}S]-GTPγS BINDING ASSAY CONDITIONS" below. Bound radioactivity was determined by filtration and scintillation counting.

SPECIFICATIONS: 1 unit=10 μg
B_{max}: 15 pmol/mg;
K_d: 7 nM for SR141716A

Species: Human CB_1 (Accession number X54937)

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

RECOMMENDED RADIOLIGAND 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 (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. Ligands were diluted in binding buffer containing 30% DMSO, then added to membranes such that the final DMSO concentration was 15%.

Radioligand: [^{3}H] SR141716A (Amersham #TRK1028)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4°C.

One vial contains enough membranes for at least 200 assays (units), where an unit is the amount of membrane that will yield approximately 4-fold signal:background with [^{3}H] SR141716A at 2 nM

[^{35}S]-GTPγS BINDING ASSAY CONDITIONS: Membranes are permeabilized by 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 $MgCl_2$/0.5 μM GDP in a nonbinding 96-well plate. Unlabeled agonist was added to the final concentration indicated in Figure 1 (final volume 100 μL), and incubated for 30 min at 30°C. The binding reaction is transferred to an FB filter plate (Millipore MAHF B1H) previously prewetted with water. The plate is washed 3 times (1 mL per well per wash) with cold 10 mM sodium phosphate, pH 7.4, then dried and counted.

PRESENTATION:

Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane protein was 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.

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

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

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