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Lot No. MLR-299A

Product Information

Cat. No. C-243

CB₂ CANNABINOID RECEPTOR, HUMAN (CHO-K1)

A frozen aliquot of membranes from CHO-K1 cells transfected with the human recombinant CB₂ cannabinoid receptor.

- Storage:** Store tightly sealed at -80°C. Long term stability of this product is being evaluated. Repeated freeze-thaw of this product is not recommended.
- Physical Properties:** Membranes are suspended in 0.1 ml 10 mM HEPES at pH 7.4, 1 mM EDTA, 1 mM MgCl₂ and 10% sucrose.
- Specific Activity:** Lot No. MLR-299A contains 3.0 pmoles/mg protein. Activity is determined by non-linear regression saturation binding analysis.
- Concentration:** 9.78 mg protein/ml.

Recommended Assay Conditions

Incubation buffer: 50 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 2.5 mM EDTA and 0.5 mg/ml BSA.

Binding Protocol:

1. Membranes: Dilute in incubation buffer (0.1 ml of membranes to 1.9 ml of incubation buffer).
2. Assay mixture: 40 µl of diluted membranes
50 µl of [³H]-radioligand in buffer
50 µl of incubation buffer or unlabeled ligand in buffer
60 µl of incubation buffer

Radioligand: [³H]-CP55940 at a final concentration of 0.34 nM for competition studies.

Unlabeled ligand: WIN 55,212-2 (Cat. No. W-102) at a final concentration of 20 µM.

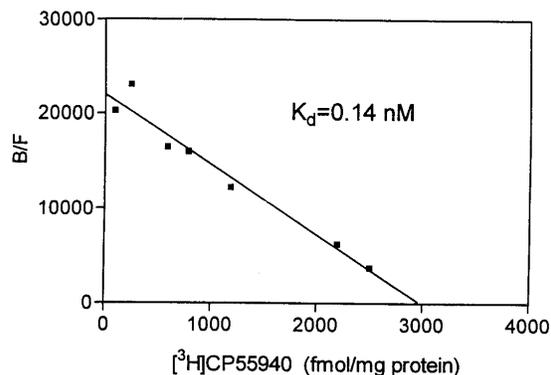
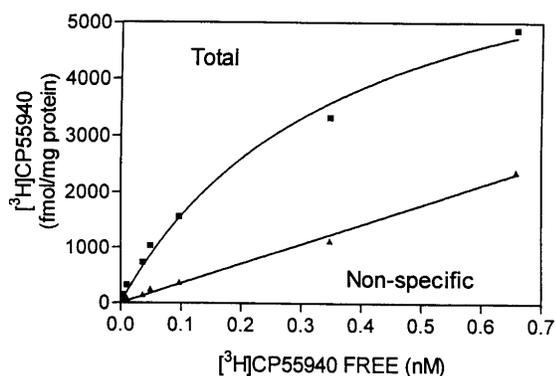
3. Incubation time: 90 minutes at 30°C.
4. Separation: Over Whatman GF/C filter (presoaked in 0.05% polyethyleneimine, Cat. No. P-182), wash 7x with 0.5 ml ice cold incubation buffer.

(OVER)

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Cat. No. C-243 (continued)

Saturation Curve: The following binding curves were obtained using Lot No. MLR-299A. The procedure used is described above.



Affinity Constants: Using a preparation of the CB₂ cannabinoid receptor, human, the following affinity constants were obtained:

LIGAND	K _i (nM)
[³ H]-CP55940	0.14 (K _d)
WIN 55,212-2 (W-102)	1.3
Anandamide (A-176)	89

Disposal: Dissolve or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber. OBSERVE ALL LOCAL, STATE AND FEDERAL LAWS.

References:

1. Devane, W.A. "New dawn of cannabinoid pharmacology." *Trends Pharmacol. Sci.* **15**, 40-41 (1994).
2. Felder, C.C. et al. "Comparison of the pharmacology and signal transduction of the human cannabinoid CB₁ and CB₂ receptors." *Mol. Pharmacol.* **48**, 443-450 (1995).
3. Gerard, C.M. et al. "Molecular cloning of a human cannabinoid receptor which is also expressed in testis." *Biochem. J.* **279**, 129-134 (1991).
4. Munro, S. et al. "Molecular characterization of a peripheral receptor for cannabinoids." *Nature* **365**, 61-65 (1993).

The specific performance characteristics of this product have not been fully investigated. RBI makes no warranty, expressed or implied, regarding suitability for any other purpose.