

## INNOPROT STABLE RECOMBINANT GPCR CELL LINES

### HUMAN CB<sub>1</sub> CANNABINOID RECEPTOR STABLE CELL LINE

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<b>Product Name:</b>	CNR1/HEK293
<b>Also Known As:</b>	CB1, CNR, CB-R, CB1A, CB1R, CANN6, CB1K5, CNR1
<b>DNA Accession Number:</b>	GenBank NM_016083
<b>Host Cell:</b>	HEK293
<b>Format:</b>	1 cryopreserved vials
<b>Quantity:</b>	> 3 x 10 <sup>6</sup> cells / vial
<b>Storage:</b>	Liquid Nitrogen

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#### **Background**

**Cannabinoid receptor.** CNR1 gene encodes a protein that is one of two cannabinoid receptors. The cannabinoids, principally delta-9-tetrahydrocannabinol and synthetic analogs, are psychoactive ingredients of marijuana. The cannabinoid receptors are members of the family of guanine-nucleotide-binding protein (G-protein) coupled receptors which inhibit adenylate cyclase activity in a dose-dependent, stereoselective and pertussis toxin-sensitive manner. The two receptors have been found to be involved in the cannabinoid-induced CNS effects (including alterations in mood and cognition) experienced by users of marijuana. Two transcript variants encoding different isoforms have been described for this gene. (Ref: Entrez Gene - CNR1 cannabinoid receptor 1).

#### **Material Provided**

Innoprot provides two vials of stably transfected cryopreserved HEK293 Cells expressing recombinant human Cannabinoid receptor 1 (GeneBank Accession Number: NM\_016083). Each vial contains > 3 x10<sup>6</sup> viable cells post-thawed.

#### **Applications**

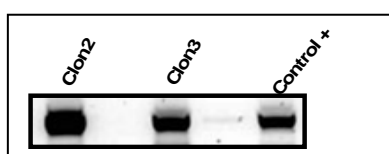
- Radioligand binding assays
- Functional assays

#### **Quality Controls**

All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Innoprot guarantees stable expression for many generations and provides support for cell culture and visualization.

## Characterization

Our expression plasmid containing the coding sequence of human Cannabinoid CN1 receptor (CNR1) was transfected in HEK293 cells, using calcium phosphate method. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).



**Fig.1. Clones CNR1 mRNA expression.**

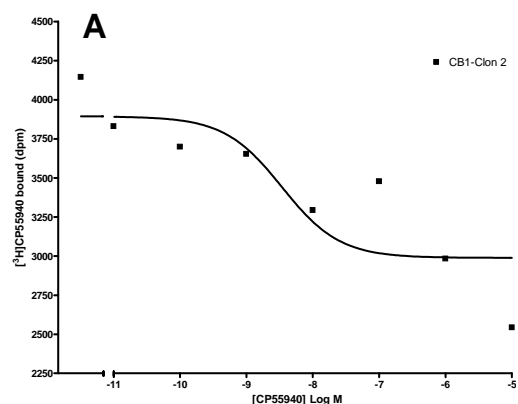
**IC50: 3,44 nM**

**pKd= 8,46**

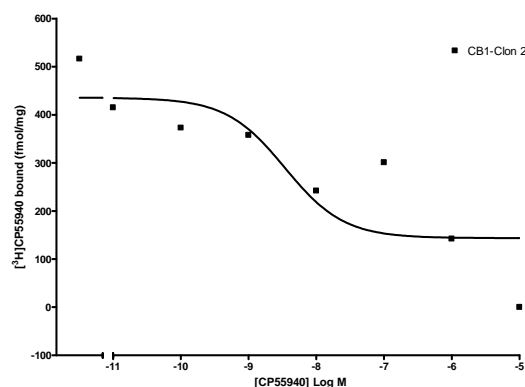
**Membrane receptor-Bmax= 3009,3 fmol/mg**

## Binding assay

**Clon2** (26 ug protein/well) was assayed with [<sup>3</sup>H]CP55940 (0,5 nM) and increasing concentrations of no radioactive competitor.

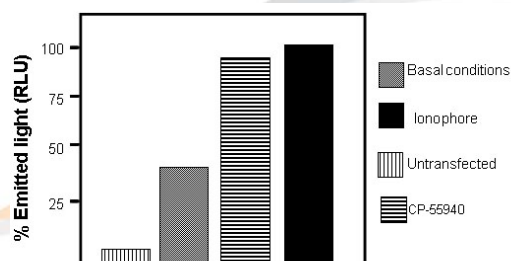


**Fig.2 competition binding assay curve**



**Fig.3. Receptor membrane density assay curve**

The positive clones were functionally tested in calcium responses. Clones cultured cells were transfected with a plasmid which contains the calcium ion sensor Case12. The Case12 protein allows direct measurement of changes of calcium concentration in a physiological range (Souslova et al., 2007). Binding of calcium is fast and reversible, allowing monitoring of intracellular calcium oscillations. In response to calcium concentration rise, Case 12 shows up to 12-fold increase of fluorescence brightness in living cells. The Case-12 transfected clones were stimulated with agonist (CP-55940 1uM). In the presence of agonist, Case12-fluorescence was increased 58% under basal conditions fluorescence (Fig.4).



**Fig.4. CNR1 functional assay.**