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READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT CB_1 RECEPTOR

CATALOG NUMBER:	HTS019F	**QUANTITY:**	1 vial, 1 mL
LOT NUMBER:		**CONCENTRATION:**	1 x 10^7 viable cells/mL

BACKGROUND:

Millipore's Ready-To-Assay™ Calcium-Optimized Cells are GPCR-expressing cell lines that are designed for simple, rapid calcium assays with no requirement for culturing cells. The user simply thaws the cells with maximal viability, dispenses into assay plates, and assays for calcium response the next day.

The Ready-To-Assay™ cells are derived from ChemiScreen™ calcium-optimized stable cell lines, which express the GPCR target of interest at high levels on the cell surface, in a host cell line containing high levels of the promiscuous Gα15 protein to couple the receptor to the calcium signaling pathway. The Ready-To-Assay™ cells are prepared by chemical treatment at a concentration optimized for effective growth arrest while maintaining high viability (>80%) after thawing and overnight plating. Pharmacological functionality of the Ready-To-Assay™ cells is identical to that of the originating GPCR cell line.

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. Such ligands include exogenous agonists such as Δ^9-THC, the main psychoactive component of the plant *Cannabis sativa*, and endogenous agonists such as anandamide that belong to eicosanoid family. 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 cloned human CB_1-expressing cell line is made in the Chem-1 host cells, an adherent cell line that supports high levels of recombinant CB_1 expression on the cell surface and contains high levels of promiscuous G protein to couple the receptor to the calcium signaling pathway. The untreated human CB_1-Chem-1 cell line and the Ready-To-Assay™ human CB_1 cells have similar EC50s for CP55940.

APPLICATIONS:

Calcium flux assay

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SPECIFICATIONS:

	EC50 for CP55940 (uM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	1.75	2914	0.54
Continuous Passage Cells	0.43	2900	0.55

HOST CELLS: Chem-1 an adherent cell line expressing a recombinant promiscuous G-protein.

TRANSFECTION: Full-length human CB_1 cDNA (Accession Number: X54937)

PLATING MEDIA:
DMEM with 4.5 g/L glucose and 4 mM glutamine (Millipore SLM-020-A)
10% heat-inactivated FBS
1x Nonessential amino acids (from 100x stock, Millipore TMS-001-C)
10mM HEPES (from 1 M HEPES, Millipore TMS-003-C)
100 U/mL Pen-Strep (from 100x stock, Millipore TMS-AB2-C)

PRESENTATION:

Cells are frozen at 1 x 10^7 cells/mL in DMEM/20% fetal bovine serum/100 U/ml penicillin and streptomycin/10% DMSO.

STORAGE: Place cells in liquid nitrogen immediately upon receipt. Maintain frozen in liquid nitrogen for up to 5 years.

ASSAY PROTOCOL:

1) Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
2) Transfer contents of the vial to a sterile 15 mL conical tube. Add 10 mL prewarmed plating media to the cells and mix gently to resuspend cells. Centrifuge at 200 x g. Remove all but 0.5 mL media.
3) Resuspend cells to 0.5 x 10^6 cells/mL in plating media. Dispense the cell suspension into a 96-well assay plate at 200 µL per well to obtain a density of approximately 1 x 10^5 cells/well.
4) Place the assay plate in a humidified 37°C incubator with 5% CO_2.
5) The cells may be assayed 16-24 hours after plating. It is recommended to wash the cells with assay buffer at least once prior to addition of loading dye.

REFERENCES:

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

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READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT CB1 RECEPTOR

Product No. HTS019F

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