

**READY-TO-ASSAY™ CALCIUM-OPTIMIZED CELLS
HUMAN RECOMBINANT OX₂ OREXIN RECEPTOR**

CATALOG NUMBER:	HTS045F	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.

The orexin/hypocretin peptides orexin A and orexin B (also known as hcrt-1 and hcrt-2) are 33- and 28-amino acid peptides, respectively, that are preferentially expressed in hypothalamus. The orexins bind to two GPCRs, OX₁ and OX₂, to increase intracellular calcium. Orexins function to increase wakefulness, primarily through OX₂, which has been shown to harbor the causative mutation in narcoleptic dogs (Lin *et al.*, 1999). In addition, the orexinergic system plays a role in feeding and stress responses (Kukkonen *et al.*, 2002). Millipore's cloned human OX₂-expressing cell line is made in the Chem-1 host cells, an adherent cell line that supports high levels of recombinant OX₂ 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 OX₂-Chem-1 cell line and the Ready-To-Assay™ human OX₂ cells have equivalent EC50s for Orexin B.

APPLICATIONS: Calcium flux assay

SPECIFICATIONS:

	EC50 for Orexin B (nM)	Maximum Signal (RFU)	Z'
Ready-To-Assay Cells	0.31	4759	0.83
Continuous Passage Cells	0.39	4544	0.81

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

TRANSFECTION: Full-length human HCRTR2 cDNA encoding OX₂ (Accession Number: NM_001526)

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 1×10^7 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 37°C 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 0.5×10^6 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 1×10^5 cells/well.
- 4) Place the assay plate in a humidified 37°C incubator with 5% CO₂.
- 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:

Kukkonen JP *et al.* (2002) Functions of the orexinergic/hypocretinergic system. *Am. J. Physiol. Cell Physiol.* 283: C1567-C1591.

Lin L *et al.* (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98: 365-376.

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HUMAN RECOMBINANT OX₂ OREXIN RECEPTOR**

Product No. HTS045F

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