Genomeditech (Shanghai) Co., Ltd.
Order: +86 021-50432825/50432826
Email: service@genomeditech.com

Product Sheet

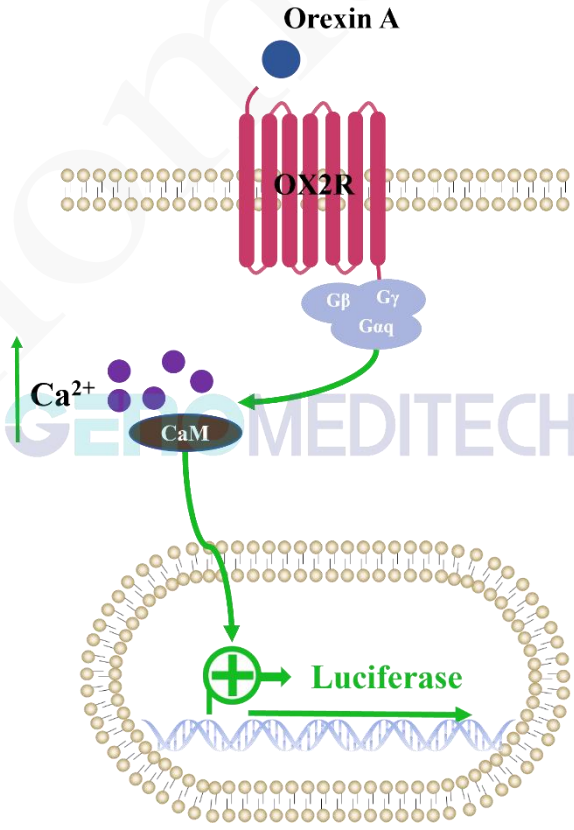
H_OX2R Reporter CHO-K1 Cell Line

Catalog number: GM-C47779

Version 4.0.26.1112

OX2R (Orexin receptor type 2, also known as hypocretin receptor 2 or HCRTR2) is a G protein-coupled receptor (GPCR) primarily expressed in the brain, particularly in the hypothalamus, as well as in certain peripheral tissues. The OX2R receptor is involved in physiological and pathological processes, including regulation of sleep-wake cycles, feeding behavior and energy metabolism, reward processing, and autonomic nervous system activity. It specifically recognizes and binds to its cognate ligands, the neuropeptides orexin-A and orexin-B (hypocretin-1 and hypocretin-2). OX2R is associated with narcolepsy, sleep-wake disorders, obesity, and metabolic dysregulation. Additionally, this receptor is implicated in mood regulation and cardiovascular control, making it a potential target of medical interest in sleep disorder treatment, metabolic disease research, and neuropsychiatric drug development.

H_OX2R Reporter CHO-K1 Cell Line is a clonal stable cell line generated by lentiviral technology, constitutively expressing human OX2R with a Gq-coupled luciferase reporter. Orexin-A/orexin-B or agonist binding activates the Gq pathway and induces luciferase expression. Luciferase activity reflects pathway activation and enables in vitro evaluation of OX2R-targeting compounds.



Specifications

Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	F12K+10% FBS+1% P.S
Growth medium	F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+4 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO_2
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
F12K	BOSTER/PYG0036
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
96 well round cell culture plate	PakGent/CL-PT096
96-well White Opaque Plate	Thermo/236108
96-well U-bottom Plate	Saining/1014010
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513
Orexin A	MCE/HY-106224
Human Orexin R2/HCRTR2 Antibody	R&D/MAB5246
SpectraMax® L Microplate Luminometer	Moleculardevices/SpectraMax L
Countess 3 Automated Cell Counter	ThermoFisher/A49891

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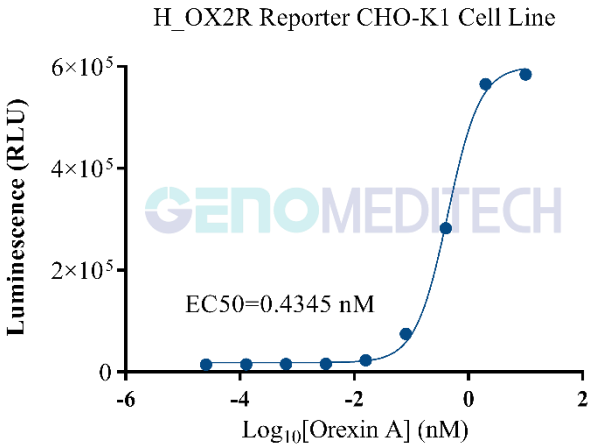
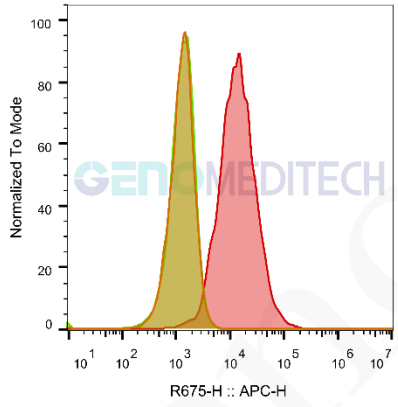
Data

Signaling Pathway



Response to Orexin A. H_OX2R Reporter CHO-K1 Cell Line (Cat. GM-C47779) was seeded at a density of 1E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Orexin A (MCE/HY-106224)) were added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [37.2]. Data are shown by drug molar concentration.

Expression



SampleID	Geometric Mean : R675-H
CHO-K1 Human Orexin R2+APC 2nd Ab	1301
H_OX2R Reporter CHO-K1 Anti-M_IgG+APC 2nd Ab	1283
H_OX2R Reporter CHO-K1 Human Orexin R2+APC 2nd Ab	13782

H_OX2R Reporter CHO-K1 Cell Line (Cat. GM-C47779) was determined by flow cytometry using Human Orexin R2/HCRTR2 Antibody (R&D/MAB5246).

Cell Recovery

Recovery Medium: F12K+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately 176 x g for 5 minutes. Discard supernatant.
d) Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.
b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
c) Aliquot 1 mL into each vial.
d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+4 μg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

a) Remove and discard culture medium.
b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
c) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 3 minutes at 37°C).
d) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
g) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 - 1:5 is recommended

Medium Renewal: Every 2 to 3 days

Notes

a) After the stabilization of the cell condition, there will be fewer dead cells post-passage, the cell growth rate will tend to stabilize, cell morphology will become uniform, and the cells will appear robust.

Related Products

HCRTR2(OX2R)	
Tango-H_OX2R CHO-K1 Cell Line	

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By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:

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