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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

OX40 / NF-κB Reporter – HEK293 Recombinant Cell Line

Catalog #: 60482

Product Description

Recombinant HEK293 cell expressing firefly luciferase gene under the control of NF-κB response elements with constitutive expression of human OX40 (Tumor Necrosis Factor Receptor Superfamily Member 4, TNFRSF4, and CD134, GenBank Accession No. NM_003327)

Background

Tumor necrosis factor receptor superfamily, member 4 (TNFRSF4), also known as CD134 and OX40, is a member of the TNFR-superfamily of receptors. OX40 is a secondary co-stimulatory immune checkpoint molecule, expressed after 24 to 72 hours following activation. This receptor has been shown to activate NF-kappaB through its interaction with adaptor proteins TRAF2 and TRAF5. Its ligand, OX40L, binds to OX40 receptors on T-cells, preventing them from dying and subsequently increasing cytokine production. OX40 has a critical role in the maintenance of an immune response beyond the first few days and onwards to a memory response due to its ability to enhance survival.

Application

- Screen for activators or inhibitors of OX40 signaling in a cellular context
- Characterize the biological activity of OX40 and its interactions with ligands

Format

Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 1A (BPS Cat. #79528): Thaw Medium 1 (BPS Cat. #60187) plus 400 μg/ml of Geneticin (Life Technologies #11811031) and 100 μg/ml of Hygromycin B (Life Technologies #10687-010).

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Cells should be grown at 37° with 5% CO_2 using **Growth Medium 1A.**

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 **(no Geneticin and Hygromycin B),** Spin down cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin and Hygromycin B).** Transfer the resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C. At first passage, switch to Growth Medium 1A (**contains Geneticin and Hygromycin)**. Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from the culture vessel with 0.05% Trypsin/EDTA. Add 6 ml of Growth Medium 1A and transfer to a tube, spin down the cells, then resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 weekly or twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (**no Geneticin)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~$2x10^6$ cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells for future use at early passage.

Materials Required but Not Supplied

- Assay Medium: Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1A (BPS Cat. #79528)
- OX40L, His tag: BPS bioscience #71185
- Anti-OX40 antagonist antibody: BPS Bioscience Cat. #72063
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS bioscience # 60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

Assay Protocol

1. Harvest OX40 / NF-κB Reporter – HEK293 cells from culture in Growth Medium 1A and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 90 µl of assay medium. Incubate the plate at 37°C in a CO_2 incubator overnight.

2. Dilute the OX40L and anti-OX40 antibody in assay medium. Add 10 µl of diluted OX40L and anti-OX40 antibody to the wells.

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Add 10 µl of assay medium to control wells.
Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.

3. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

4. Perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.
If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

5. Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 1. Dose Response of OX40 / NF-κB Reporter – HEK293 cells to OX40L.
The results are shown as fold induction of NF-κB luciferase reporter expression.

The EC50 of OX40L is 67.87 ng/ml.

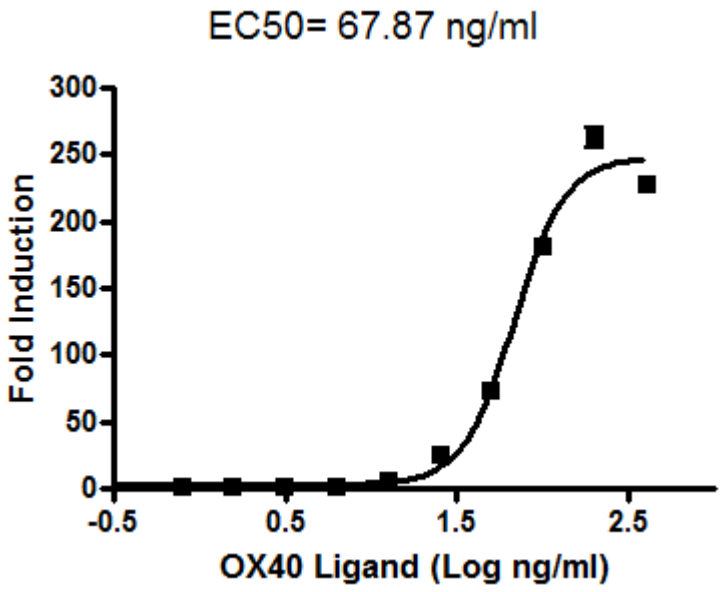

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Figure 2. Anti-OX40 antagonist antibody inhibition dose response in OX40 / NF-κB Reporter – HEK293 cells
Cells were treated with anti-OX40 antagonist antibody and 400 ng/ml of OX40L simultaneously for 6 hours.
The IC50 of anti-OX40 antagonist antibody is 0.61 µg/ml

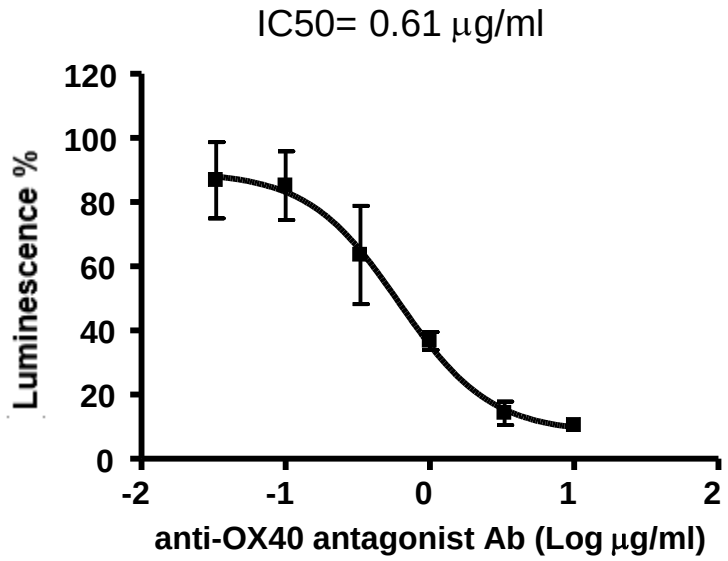
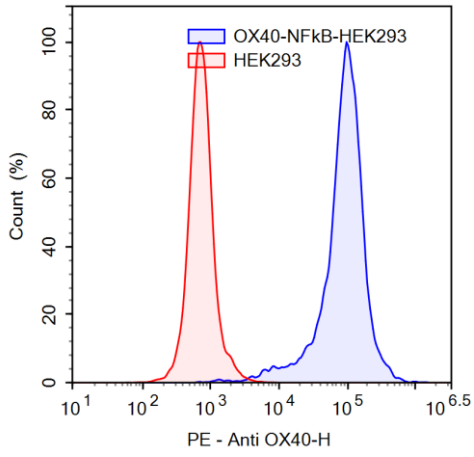


Figure 3. FACS analysis of OX40 in HEK293 cells
OX40-HEK293 cells were stained with PE-labeled anti-OX40 antibody and analyzed by FACS. Y-axis shows the cell count. X-axis shows the intensity of PE fluorescence.



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Sequence

hOX40 sequence (accession number NM_003327)

MCVGARRLGRGPCAALLLLGLGLSTVTGLHCVGDTYPSNDRCCHECRPGNGMVSRCSRSQNTVC
RPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLCTATQDTVCRCRAGTQPLDSYKPGVDCAPC
PPGHFSPGDNQACKPWTNCTLAGKHTLQPASNSSDAICEDRDPPATQPQETQGPPARPITVQPT
EAWPRTSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLPPDAHKPPGG
GSFRTPIQEEQADAHSTLAKI*

Related Products

Product	Cat. #	Size
NF-kB Reporter (Luc)-HEK293 cell line	60650	2 vials
Anti-OX40 antagonist antibody	72063	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Human OX40L (CD252), His tag	71185	100 µg
Human OX40 (CD134), Fc fusion	71175	100 µg
Human OX40 (CD134), His tag, Biotin-labeled	71310	50 µg

Notes

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