Description

The OX40/NF-κB Luciferase Reporter HEK293 Cell Line is a HEK293 cell line expressing the Firefly luciferase reporter under the control of NF-κB (nuclear factor kappa light chain enhancer of activated B cells)-responsive elements and OX-40 (Tumor Necrosis Factor Receptor Superfamily Member 4, GenBank Accession No. NM_003327).

This cell line has been validated by flow cytometry, and with OX40L and anti-OX40 antagonist antibodies.

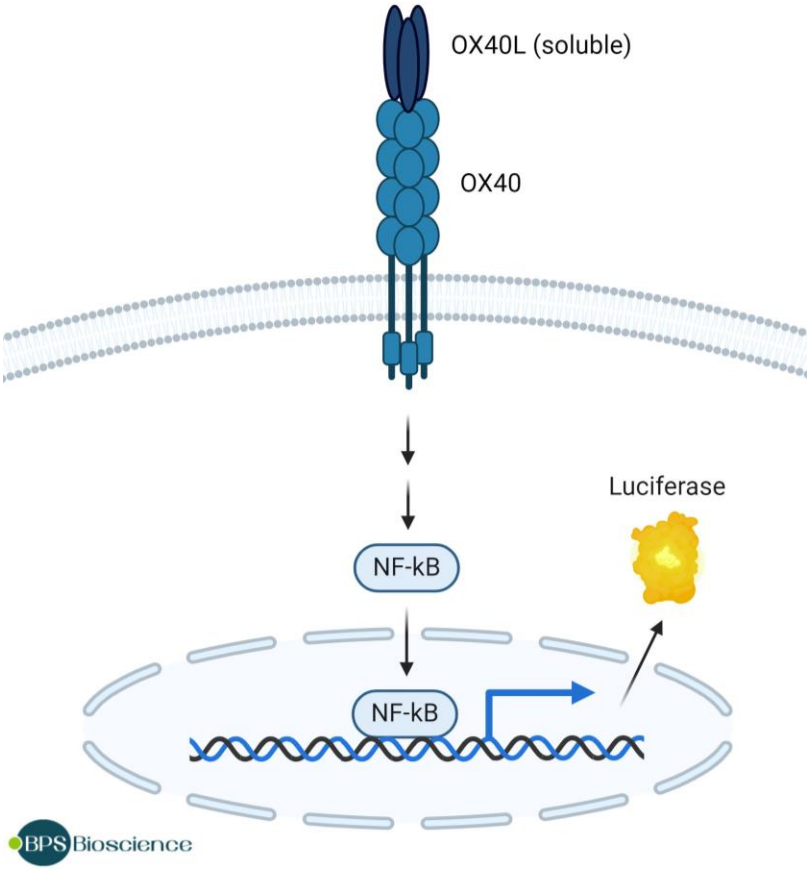

Figure 1: Illustration of mechanism of action of the OX40/NF-κB Luciferase Reporter HEK293 Cell Line.

Background

OX40, also known as CD134, is a co-stimulatory receptor, of the TNF (tumor necrosis factor) receptor family, expressed on the surface of T cells. Binding of OX40 to its ligand, OX40L (also known as CD252), potentiates T cell activation, differentiation, proliferation, survival and T cell effector function. OX40L is present in NK cells, participating in their activation and cytotoxicity profile, and dendritic cells. OX40 can bind to members of the TRAF (TNFR associated factor) family of proteins, which can then regulate the NF-κF (nuclear factor kappa-light chain enhancer of activated B cells) signaling pathway. OX40 and OX40L can be found in cancer cells, such as AML (acute myeloid leukemia) and breast cancer cells. Studies have shown that OX40 agonists can increase anti-tumor immunity and improve tumor-free survival in pre-clinical studies. Alternatively, OX40 antagonists offer potential as therapeutics for inflammatory diseases. The development of new modulators of the OX40/OX40L activity are promising therapies for patients suffering from solid tumors or auto-immune disorders.

Application

- Characterize OX40 activity and binding to ligands.
- Screen for compounds that regulate OX40 signaling in a cellular model.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528
OX40L (CD252), His-tag (Human) Recombinant	BPS Bioscience #71185
Anti-OX40 Antagonist Antibody	BPS Bioscience #72063
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1A (BPS Bioscience #79528):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 100 µg/ml Hygromycin B and 400 µg/ml G418.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1A.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

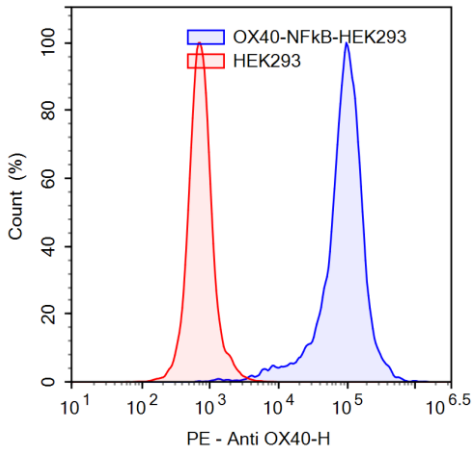

Figure 1: Cell surface expression of OX40 in OX40/NF-κB Luciferase Reporter HEK293 Cell Line. OX40/NF-κB Luciferase Reporter HEK293 cells (blue) and control HEK293 cells (red) were stained with Anti-OX40 Antibody, PE-Labeled (#72064) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

Functional Validation

- The following assays were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Treated Cells", "Luminescence Background Control" and "Untreated Control" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Seed OX40/NF-κB Luciferase Reporter HEK293 cells at a density of ~70,000 cells/well in 90 µl of Assay Medium into a clear-bottom, white 96-well plate. Leave a few empty wells as cell-free control wells ("Luminescence Background Control").

2. Incubate the plate at 37°C in a 5% CO_2 incubator for 24 hours.

3. Prepare serial dilutions of OX40L or Anti-OX40 Antagonist Antibody in Assay Medium at concentrations 10-fold higher than the desired final concentrations.

4. Add 10 µl of each diluted compound to the wells labeled as "Treated Cells".

5. Add 10 µl of Assay Medium to the wells labeled as "Untreated Control".

6. Add 100 µl of Assay Medium to the wells not containing cells and labeled as "Luminescence Background Control".

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

8. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.

9. Measure luminescence using a luminometer.

10. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of untreated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Treated\ Wells - avg.background}{Avg.Luminescence\ of\ Untreated\ Wells - avg.background}$$

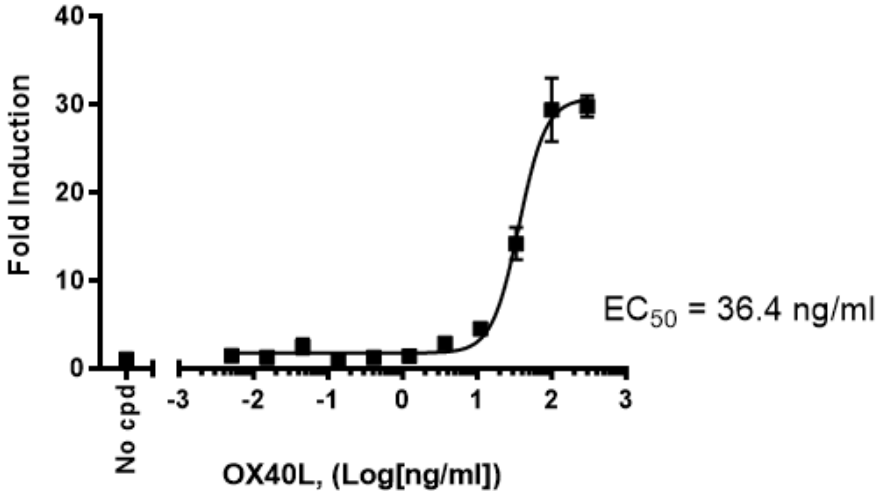

Figure 3: Dose curve response of OX40/NF-κB Luciferase Reporter HEK293 Cell Line to OX40L.
OX40/NF-κB Luciferase Reporter HEK293 cells were incubated with increasing concentrations of OX40L for 6 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells in the absence of OX40L.

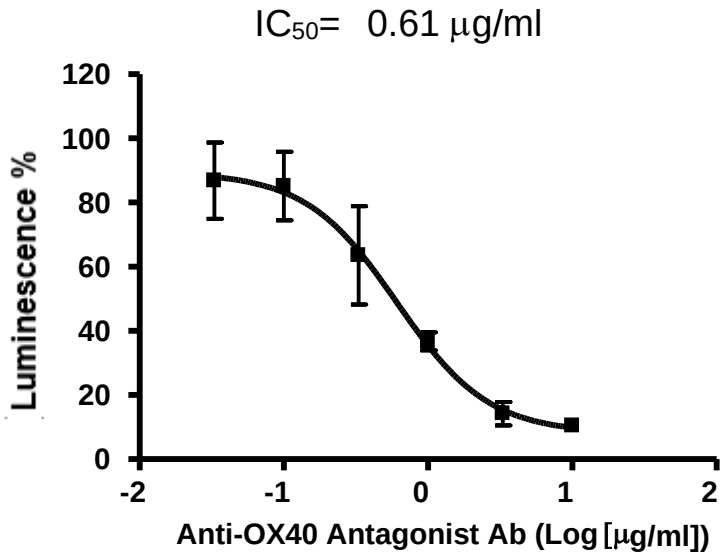

Figure 3: Dose curve response of OX40/NF-κB Luciferase Reporter HEK293 Cell Line to Anti-OX40 Antagonist Antibody, in the presence of OX40L.
OX40/NF-κB Luciferase Reporter HEK293 cells were incubated with increasing concentrations of Anti-OX40 Antagonist Antibody and 400 ng/ml of OX40L for 6 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to the activity of cells in the absence of OX40L.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence

Human OX40 sequence (NM_003327)

MCVGARRLGRGPCAALLLLGLGLSTVTGLHCVGDTYPSNDRCCHECRPGNGMVSRCSRSQNTVCRPCGPGFYNDVVSSKPCKP
CTWCNLRSGSERKQLCTATQDTVCRCRAGTQPLDSYKPGVDCAPCPPGHFSPGDNQACKPWTNCTLAGKHTLQPASNSSDAIC
EDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLALYLLRRDQRLPPDAHK
PPGGGSFRTPIQEEQADAHSTLAKI*

References

Peng K., *et al.*, 2014 *AAPS J.* 16(4): 625–633.
Marconato M., *et al.*, 2022 *Scientific Reports* 12: 15856.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
OX40 – HEK293 Recombinant Cell Line	60682	2 vials
NF-kB reporter (Luc) - Jurkat Cell line	100644	2 vials
Anti-OX40 Antagonist Antibody	40707	50 µg/100 µg
Anti-OX40 Competitive Antibody	60544	50 µg/100 µg
OX40[Biotinylated]:OX40L Inhibitor Screening Assay Kit	72045	96 reactions
Human OX40 (CD134), Biotin-labeled, His tag (Human) Recombinant	71310	25 µg/50 µg

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