

**Description**

The OX40 - HEK293 Recombinant Cell Line is a HEK293 cell line expressing human OX40 (TNF receptor superfamily member 4, GenBank Accession #NM\_003327).

This cell line has been validated by flow cytometry.

**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- $\kappa$ B (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**

Screen for compounds that bind to OX40, such as anti-OX40 antibodies, in a cellular model.

**Materials Provided**

Components	Format
2 vials of frozen cells	Each vial contains $>1 \times 10^6$ 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	<a href="#">BPS Bioscience #60187</a>
Growth Medium 1F	<a href="#">BPS Bioscience #79540</a>

**Storage Conditions**

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a  $-80^{\circ}\text{C}$  freezer for long term storage. Contact technical support at [support@bpsbioscience.com](mailto: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°C with 5% CO<sub>2</sub>. 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 1F (BPS Bioscience #79540):*

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

## 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<sub>2</sub> 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<sub>2</sub> 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 1F.

### Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca<sup>2+</sup>/Mg<sup>2+</sup>, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1F 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 1F.
4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice a week.

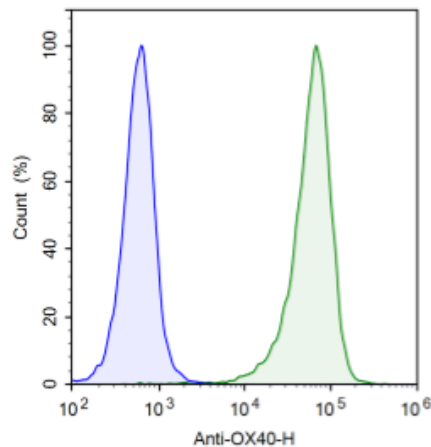
#### Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca<sup>2+</sup>/Mg<sup>2+</sup> and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.
2. Once the cells have detached, add Growth Medium 1B 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<sup>6</sup> 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





	Samples	Subset	Cell Count
	OX40-HEK293 Cell	Live Singlet	14,041
	Control HEK293 Cell	Live Singlet	14,805

Figure 1: Cell surface expression of OX40 in OX40 - HEK293 Recombinant Cell Line.

OX40 - HEK293 cells (green) and control HEK293 cells (blue) 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.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com).

**Sequence**

Human OX40 sequence (NM\_003327)

MCVGARRLGRGPCAALLLLGLGLSTVTGLHCVGDTYPSNDRCCHECRPGNGMVSRCRSRQNTVCRPCGPGFYNDVVSSKPCKP  
 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](https://bpsbioscience.com/license) for the label license and other key information about this product.**Troubleshooting Guide**Visit [bpsbioscience.com/cell-line-faq](https://bpsbioscience.com/cell-line-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com).**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
OX40 / NF-κB Luciferase Reporter HEK293 Cell Line	60482	2 vials
NF-κB 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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