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## **Data Sheet**

### **OX40 - HEK293 Recombinant Cell Line**

#### **Cat #: 60682**

#### **Product Description**

Recombinant HEK293 stably expressing human OX40 (TNF receptor superfamily member 4, TNFRSF4, ACT35; CD134; IMD16; TXGP1L, GenBank Accession #NM\_003327).

#### **Background**

OX40 (CD134) is a co-stimulatory receptor expressed on the surface of CD4+ and CD8+ T cells 24 to 48 hours after activation. Binding of OX40 to its ligand, OX40L (CD252), present on dendritic cells, potentiates T cell survival and increases cytokine production. OX40 has been shown to activate NF- $\kappa$ B-mediated memory cell generation through its interaction with adaptor proteins TRAF2 and TRAF5. OX40 has a critical role in the maintenance of an immune response beyond the first few days and onwards to a memory response.

#### **Application**

- OX40 binding molecule (such as anti-OX40 antibody) screening and profiling in a cellular context

#### **Format**

Each vial contains  $\sim 2 \times 10^6$  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 metabolite-based Mycoplasma Detection Kit (Biotool #B3903) to confirm the absence of Mycoplasma species.

#### **General Culture Conditions**

**Thaw Medium 1 (BPS Cat. #60187):** MEM medium (Hyclone #SH30024.01) + 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 1F (BPS Cat. #79540):** Thaw Medium 1 (BPS Cat. #60187) plus 100  $\mu$ g/ml of Hygromycin B (Life Technologies #10687-010).

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Cells should be grown at 37°C with 5% CO<sub>2</sub> using Growth Medium 1F to ensure recombinant expression. OX40 HEK293 cells should display a typical cell division time of about 24 hours.

**To thaw the cells**, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 (**no Hygromycin B**), spin down cells at 1000 rpm and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Hygromycin**). Transfer resuspended cells to T25 flask and culture at 37°C in a 5% CO<sub>2</sub> incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 (**no Hygromycin B**), and continue growing culture in a CO<sub>2</sub> incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 1F (**contains Hygromycin B**).

**To passage the cells**, rinse cells with phosphate buffered saline (PBS) and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1F (**contains Hygromycin B**) and transfer to a tube, spin down cells, resuspend cells in Growth Medium 1F (**contains Hygromycin B**) and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ratio: 1:5 to 1:10 weekly or twice a week.

*Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split with a higher ratio.*

**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 Hygromycin B**) and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2x10<sup>6</sup> 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.

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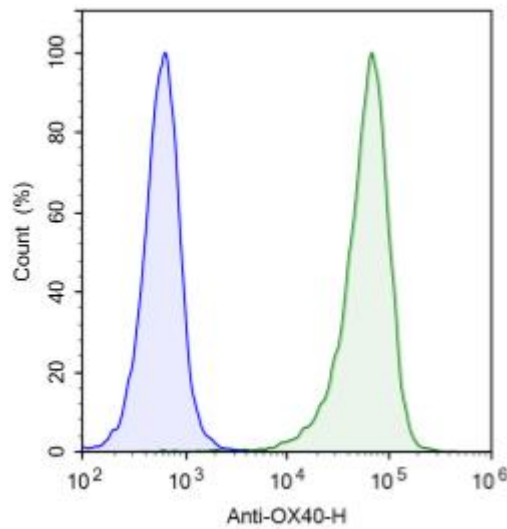
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**Validation**

Cell surface expression of human OX40 in OX40-HEK293 cells was confirmed by flow cytometry.

**Figure 1. Flow cytometry analysis of cell surface expression of OX40 in OX40-HEK293 cells.**

OX40-HEK293 cells (green) or control HEK293 cells (blue) were stained with PE-labeled Anti-OX40 Antibody (BPS Bioscience #72064) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



	<b>Samples</b>	<b>Subset</b>	<b>Cell Count</b>
	OX40-HEK293 Cell	Live Singlet	14,041
	Control HEK293 Cell	Live Singlet	14,805

**Sequence**

OX40 sequence (accession number NM\_003327)

MCVGARRLGRGPCAALLLLGLGLSTVTGLHCVGDTYPSNDRCCHECRPGNGMVSRCSRS  
 QNTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLCTATQDTVCRCRAGTQPLDS  
 YKPGVDCAPCPGPHFSPGDNQACKPWTNCTLAGKHTLQPASNSSDAICEDRDPATQPQ  
 ETQGPPARPI TVQPTEAWPRTSQGPSTRPVEVPGGRAVAAILGLGLVLGLLGPLAILLA  
 LYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI

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## Related Products

<b>Product</b>	<b>Cat. #</b>	<b>Size</b>
OX40 / NF-κB Reporter – HEK293 Recombinant Cell Line	60482	2 vials
NF-κB reporter (Luc) - Jurkat Cell line	60651	2 vials
NF-κB Reporter Kit	60614	500 reactions
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
Anti-OX40 Antibody, PE-labeled	72064-2	100 µg
Anti-OX40 Antagonist Antibody	72063-2	100 µg
Thaw Medium 1	60187	100 ml
OX40 Screening & Profiling		

## Notes

License Disclosure: Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit the use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees; contact [sales@bpsbioscience.com](mailto:sales@bpsbioscience.com) for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

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