Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

Human OX40 (Luc) HEK293 Reporter Cell

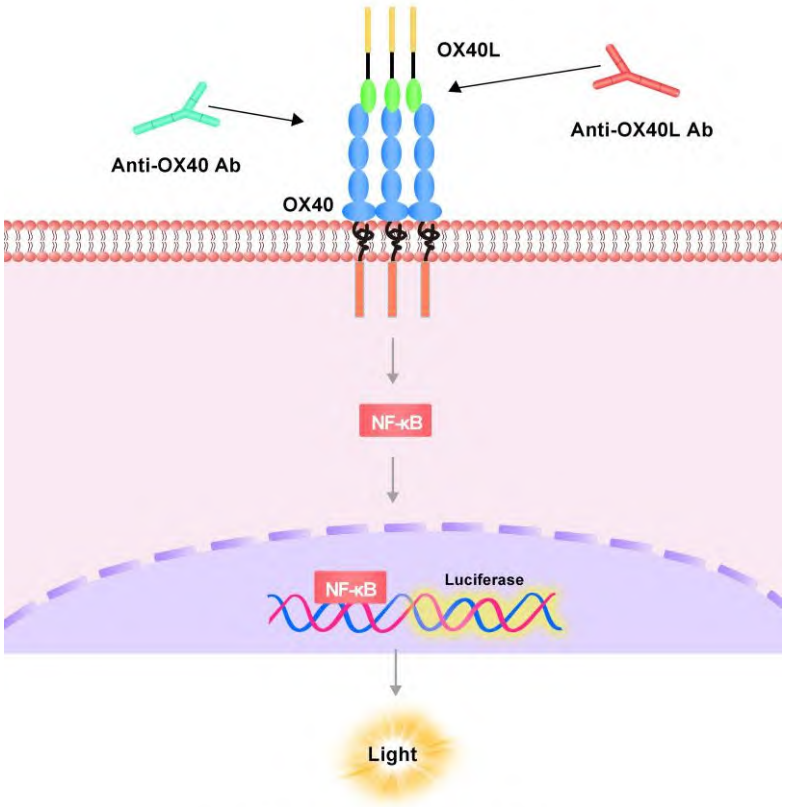
Catalog No.	Size
CHEK-ATF135	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human OX40 (Luc) HEK293 Reporter Cell was engineered to not only express NF-κB signaling response element, but also express the receptor full length human OX40 (Gene ID: 7293), which can drive luciferase expressing systems by OX40 ligand/agonist antibody stimulation. When stimulated with human OX40 ligand protein, the OX40 ligand/OX40 interaction drives NF-κB-mediated luminescence. Inhibition of OX40 ligand binding to OX40 by either anti-OX40 ligand or anti-OX40 antibodies results in a decrease in luminescence.

• *Application*

- Screen for anti-human OX40 ligand or anti-human OX40 neutralizing antibody.
- Screen for ligands or agonist antibodies that can bind and activate OX40.



Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

• *Cell Line Profile*

Cell line	Human OX40 (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin (20 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin (20 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

• *Recovery*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
4. Resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37℃ with 5% CO_2 incubator until the cells are ready to be split.

• *Subculture*

1. Remove and discard culture medium.
2. Wash the cells once with sterile PBS.
3. Add 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached.
4. Add 6.0 to 8.0 mL of culture medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessel.
6. Incubate at 37℃ with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended.

Medium Renewal: Every 2 to 3 days.

Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

• *Cryopreservation*

1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with culture medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transferring to liquid nitrogen storage.

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

- ***Receptor Assay***

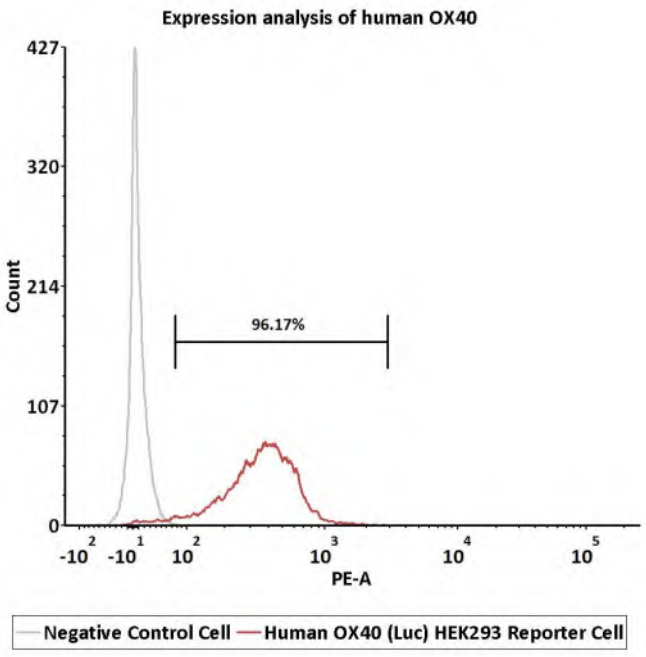


Fig1. Expression analysis of human OX40 on Human OX40 (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human OX40 (Luc) HEK293 Reporter Cell or negative control cell using biotinylated human OX40 ligand protein followed by staining with Streptavidin-PE.

Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

- ***Signaling Bioassay***

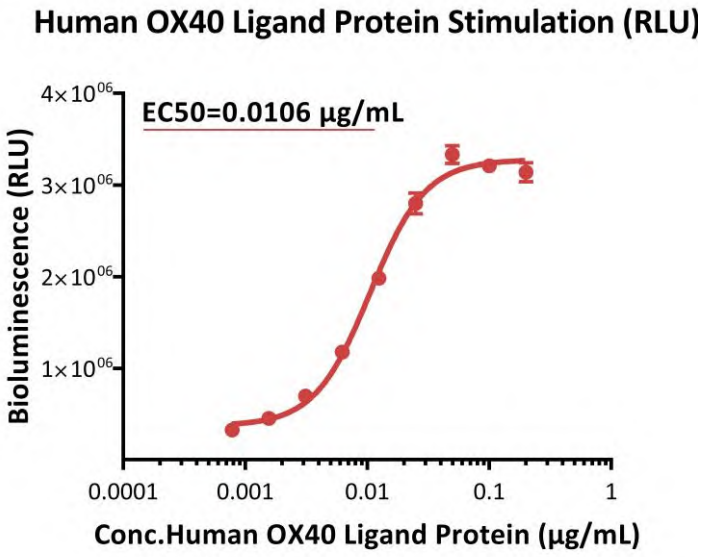


Fig2. Response to human OX40 ligand protein (RLU). The Human OX40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human OX40 ligand protein. The EC50 was approximately 0.0106 μg/mL.

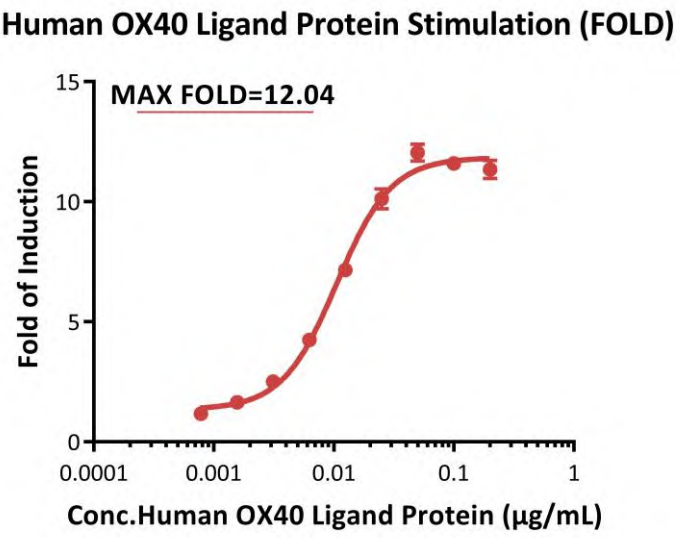


Fig3. Response to human OX40 ligand protein (FOLD). The Human OX40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human OX40 ligand protein. The max induction fold was approximately 12.04.

Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

• *Application*

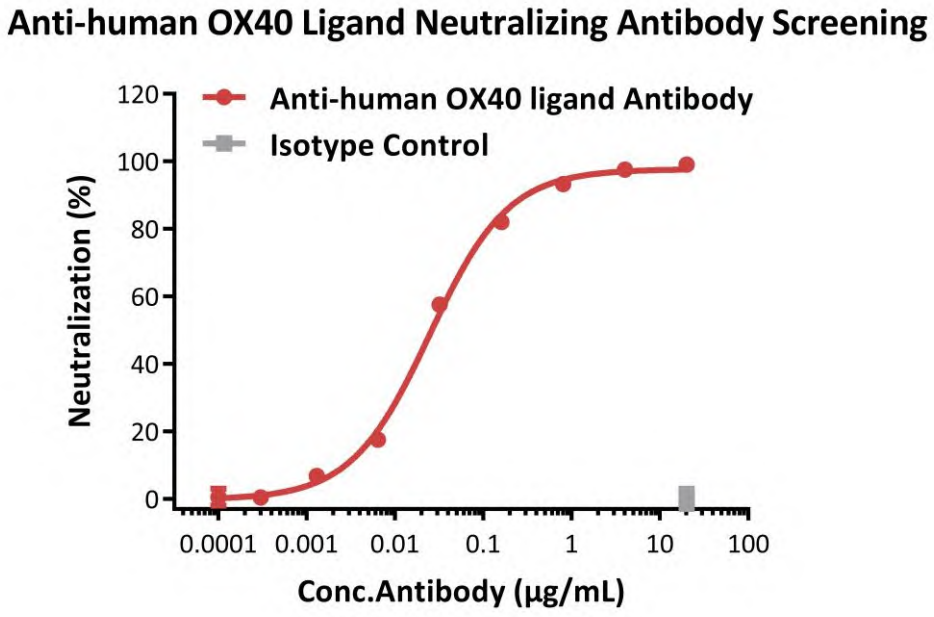


Fig4. Inhibition of human OX40 ligand protein-induced reporter activity by anti-human OX40 ligand neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human OX40 ligand protein with a final concentration of 0.02 μg/mL. The EC50 of anti-human OX40 ligand neutralizing antibody (Amlitelimab) was approximately 0.025 μg/mL.

• *License Disclosure*

This reporter cell is provided for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make this reporter cell available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit modification of this reporter cell in any way. Inappropriate use or distribution of this reporter cell will result in revocation of the license. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees. AcroBiosystems does not warrant the suitability of this reporter cell for any particular use, and does not accept any liability in connection with the handling or use of this reporter cell.

Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

• *Related Products*

Products	Cat.No.
Human CD40 (Luc) HEK293 Reporter Cell	CHEK-ATF097
Human 4-1BB (Luc) HEK293 Reporter Cell	CHEK-ATF073