# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

## ➢ *Limited Use & License Disclosure*

**BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE FOLLOWING TERMS OF LIMITED USE OF THIS CELL LINE PRODUCT.**

- If the researcher is not willing to accept the terms of limited use of this cell line product, and the product is unused, ACRO will accept return of the unused product.
- Researchers may use this product for research use only, no commercial use is allowed. "Commercial use" means any and all uses of this product and derivatives by a party for profit or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research.
- This cell line is neither intended for any animal or human therapeutic purposes nor for any direct human in vivo use. You have no right to share, modify, transfer, distribute, sell, sublicense, or otherwise make the cell line available for use to other researchers, laboratories, research institutions, hospitals, universities, or service organizations.
- ACROBIOSYSTEMS MAKES NO WARRANTIES OR REPRESENTATIONS OF ANY KIND, EITHER EXPRESSED OR IMPLIED, WITH RESPECT TO THE SUITABILITY OF THE CELL LINE FOR ANY PARTICULAR USE.
- ACROBIOSYSTEMS ACCEPTS NO LIABILITY IN CONNECTION WITH THE HANDLING OR USE OF THE CELL LINE.
- Modifications of the cell line, transfer to a third party, or commercial use of the cell line may require a separate license and additional fees. Please contact order.cn@acrobiosystems.com for further details.

# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

## Human OX40 (Luc) HEK293 Reporter Cell

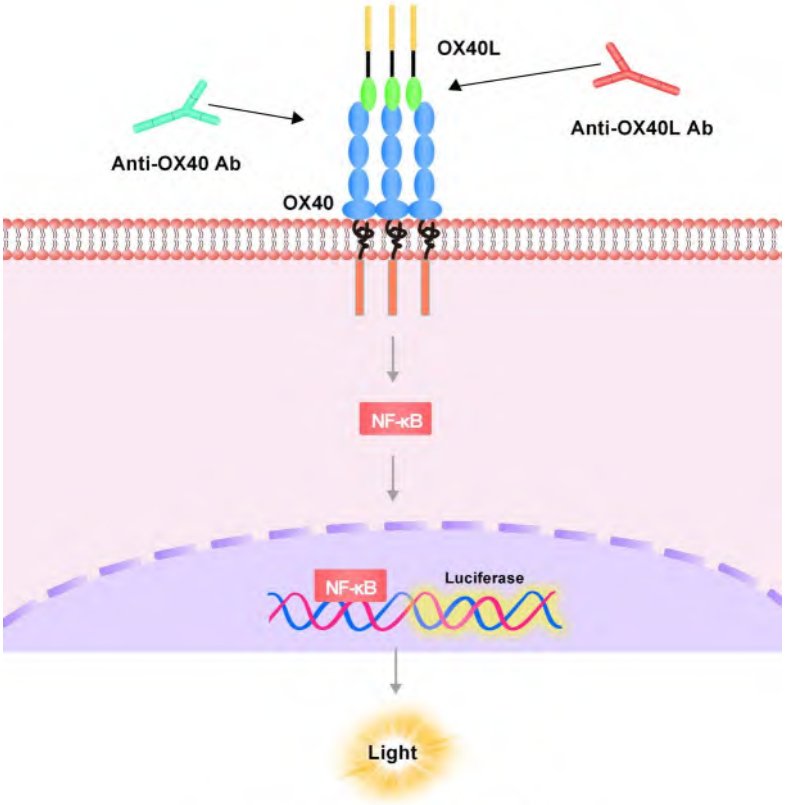
| Catalog No. | Size |
|---|---|
| CHEK-ATF135 | 2 × (1 vial contains ~5×10^6 cells) |

### • *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 (Uniprot: P43489), 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 B (20 μg/mL) |
| **Incubation** | 37℃ with 5% $CO_2$ |
| **Doubling Time** | 22-24 hours |
| **Transduction Technique** | Lentivirus |

## • *Materials Required for Cell Culture*

- DMEM Medium (BasalMedia, Cat. No. L120KJ)

**Note:** If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ) in China, you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

**Note:** For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS, 1%P/S
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin B (20 μg/mL), Zeocin (20 μg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- $CO_2$ Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

## • *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37°C with 5% $CO_2$ incubator until the cells are ready to be split.

## • *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:4 to 1:8 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% $CO_2$ incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

**Note:** After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

## • *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of $5\times10^6$ to $1\times10^7$ cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

**Note:** It is recommended to establish a cell bank at the earliest possible passage for long-term use.

## • *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

- ***Receptor Assay***

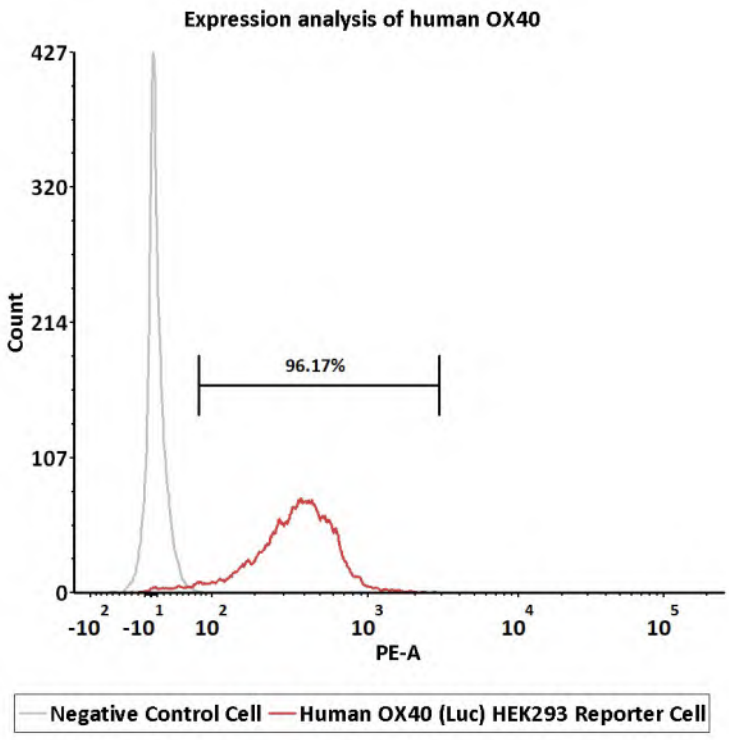



**Fig1. Expression analysis of human OX40 on Human OX40 (Luc) HEK293 Reporter Cell by FACS.** Cell surface staining was performed on Human OX40 (Luc) HEK293 Reporter Cell or negative control cell using biotinylated human OX40 ligand protein followed by staining with Streptavidin-PE.

# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

- ***Signaling Bioassay***

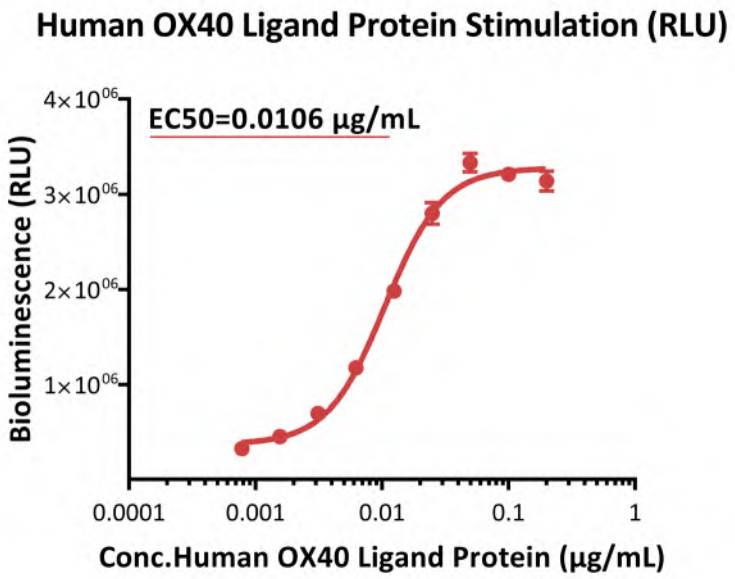



**Fig2. Response to human OX40 ligand protein (RLU).** The Human OX40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human OX40 ligand protein. The EC50 was approximately 0.0106 μg/mL.

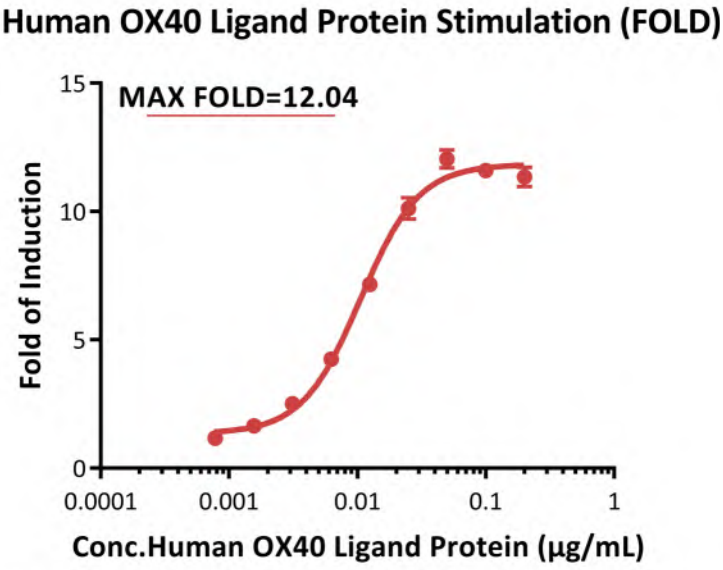



**Fig3. Response to human OX40 ligand protein (FOLD).** The Human OX40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human OX40 ligand protein. The max induction fold was approximately 12.04.

- *Application*

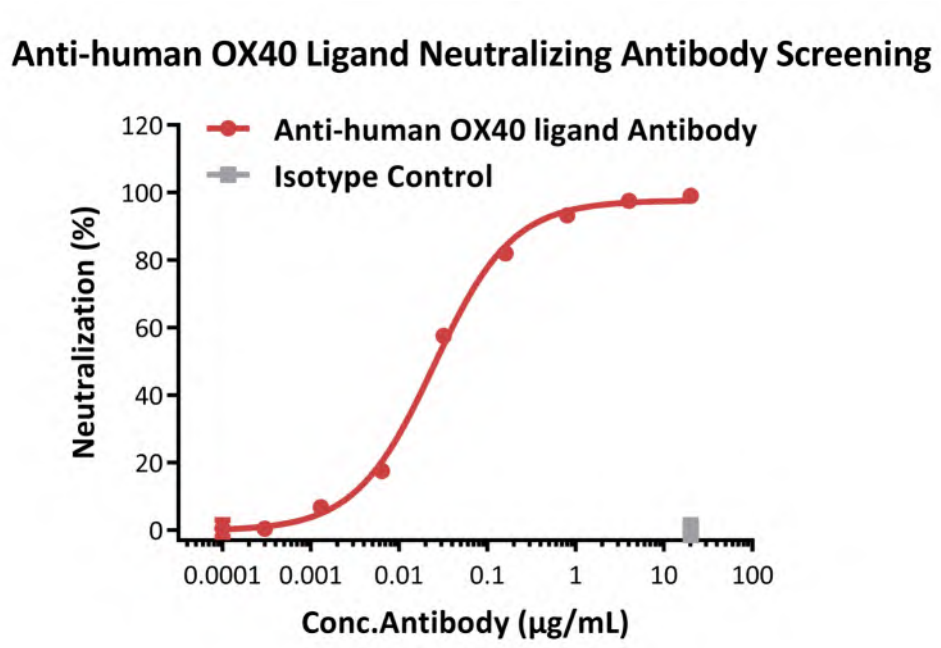



**Fig4. Inhibition of human OX40 ligand protein-induced reporter activity by anti-human OX40 ligand neutralizing antibody.** This reporter cell was incubated with serial dilutions of antibodies in the presence of human OX40 ligand protein with a final concentration of 0.02 μg/mL. The EC50 of anti-human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

# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

## • *Related Products*

| Products | Cat.No. |
|---|---|
| HEK293/Membrane-Bound human TL1A Stable Cell Line | CHEK-ATP198 |
| Raji/Membrane-Bound Human TL1A Stable Cell Line Development Service | SCRAJ-STT204 |
| HEK293/Human TL1A Stable Cell Line | CHEK-ATP142 |
| Human TSLP R (Luc) HEK293 Reporter Cell | CHEK-ATF045 |
| STAT3 (Luc) HEK293 Reporter Cell | CHEK-ATF047 |
| Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell | CHEK-ATF074 |
| HEK293/Human OX40 / TNFRSF4 / CD134 Stable Cell Line | CHEK-ATP053 |
| HEK293/Human OX40 Ligand / TNFSF4 Stable Cell Line | CHEK-ATP054 |
| HEK293/Human FcRn (FCGRT & B2M) Stable Cell Line | CHEK-ATP079 |
| Human IL-11 R alpha (Luc) HEK293 Reporter Cell | CHEK-ATF052 |
| Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell | CHEK-ATF075 |
| Human IL-21 R/CD132 (Luc) HEK293 Reporter Cell | CHEK-ATF051 |
| Human CD40 (Luc) HEK293 Reporter Cell | CHEK-ATF097 |
| Human IL-10 R alpha/IL-10 R beta (Luc) HEK293 Reporter Cell | CHEK-ATF095 |
| Human DR3 (TL1A receptor) (Luc) Jurkat Reporter Cell Development Service | SCJUR-STF178 |
| Human IL-7 R alpha/CD132 (Luc) HEK293 Reporter Cell | CHEK-ATF099 |
| NIH-3T3/Human IGF-1 R Stable Cell Line Development Service | CNIH-ATP102 |
| Human HVEM (Luc) HEK293 Reporter Cell | CHEK-ATF105 |
| Human BTLA (Luc) Jurkat Reporter Cell Development Service | SCJUR-STF106 |
| Human IGF-1 R (Luc) HEK293 Reporter Cell | CHEK-ATF107 |
| Raji/Human HVEM Stable Cell Line Development Service | SCRAJ-STF108 |
| CHO/Human LIGHT Stable Cell Line Development Service | SCCHO-ATP109 |
| CHO/Human BTLA Stable Cell Line Development Service | SCCHO-ATP110 |
| CHO/Human TSHR Stable Cell Line Development Service | SCCHO-ATP085 |

# Human OX40 (Luc) HEK293 Reporter Cell Data Sheet

## • *Related Products*

| Products | Cat.No. |
|---|---|
| CHO/Human LILRB4 Stable Cell Line Development Service | SCCHO-ATP087 |
| Human GLP-2R (Luc) HEK293 Reporter Cell | CHEK-ATF128 |
| Human RANK (Luc) HEK293 Reporter Cell | CHEK-ATF129 |
| HEK293/FcRn (FCGRT & B2M), GFP Tag Stable Cell Line | CHEK-ATP132 |
| HEK293/Human TSHR Stable Cell Line | CHEK-ATP086 |
| HEK293/Human LILRB4 Stable Cell Line | CHEK-ATP088 |
| Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell | CHEK-ATF133 |
| Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell | CHEK-ATF094 |
| Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell | CHEK-ATF136 |
| HEK293/Human HVEM Stable Cell Line | CHEK-ATP147 |
| Human IL-23 R/IL-12 R beta 1(Luc) HEK293 Reporter Cell | CHEK-ATF166 |
| Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell | CHEK-ATF167 |
| HEK293/Human CD40 Ligand / TNFSF5 Stable Cell Line | CHEK-ATP041 |
| Human TSHR (Luc) HEK293 Reporter Cell | CHEK-ATF187 |
| Human PTH1R (Luc) HEK293 Reporter Cell | CHEK-ATF194 |
| Human TACI (Luc) HEK293 Reporter Cell | CHEK-ATF197 |