Human CD40 (Luc) HEK293 Reporter Cell Data Sheet

Human CD40 (Luc) HEK293 Reporter Cell

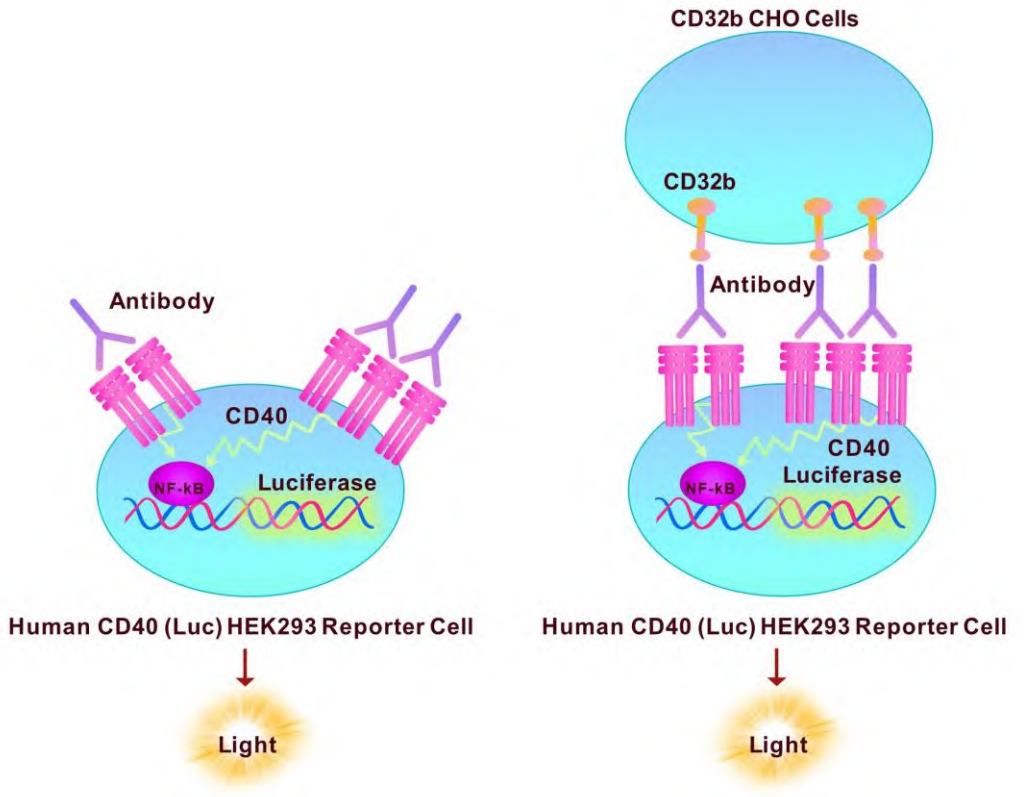
Catalog No.	Size
CHEK-ATF097	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human CD40 (Luc) HEK293 Reporter Cell was engineered to not only express NF-κB signaling response element, but also express the receptor full length human CD40 (Gene ID: 958), which can drive luciferase expressing systems by CD40 ligand/ agonist antibody stimulation. In the absence of agonist antibody or CD40 ligand, the CD40 receptor is not activated and luminescence signal is low. In the presence of agonist antibody or CD40 ligand, the CD40 pathway-activated luminescence can be detected in a dose-dependent manner. This reporter cell can also be used to test agonist antibody whether in an FcγR-dependent manner to strengthen the agonistic activity.

• *Application*

- Screen for ligands or agonist antibodies that can bind and activate CD40.



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• *Cell Line Profile*

Cell line	Human CD40 (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)

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• *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.

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• *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

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- ***Receptor Assay***

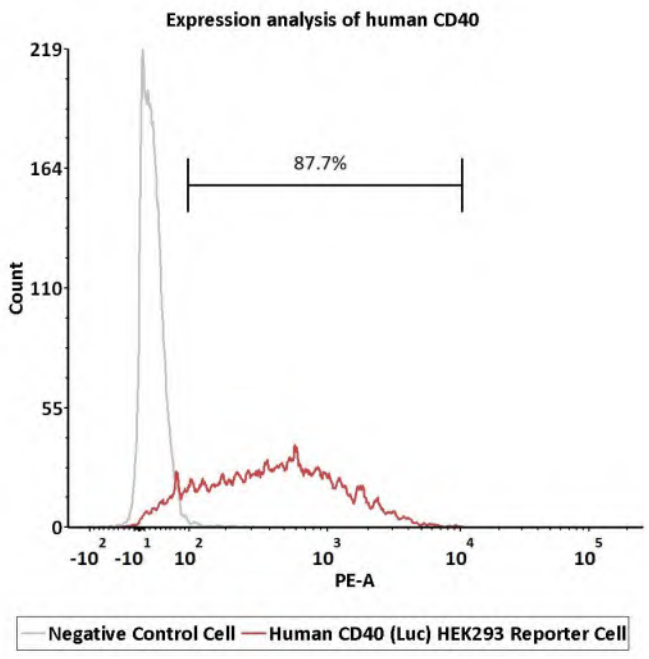

Fig1. Expression analysis of human CD40 on Human CD40 (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human CD40 (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human CD40 antibody.

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- ***Signaling Bioassay***

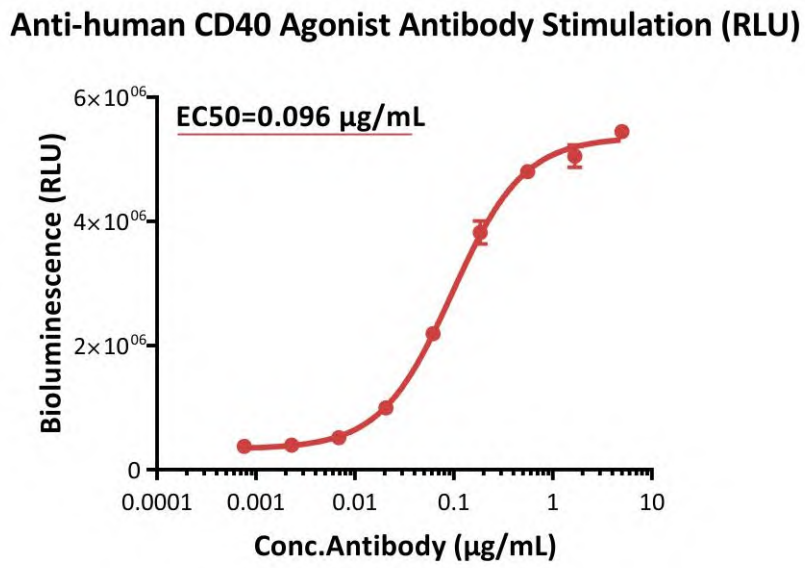

Fig2. Response to anti-human CD40 antibody (RLU). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human CD40 antibody. The EC50 was approximately 0.096 μg/mL.

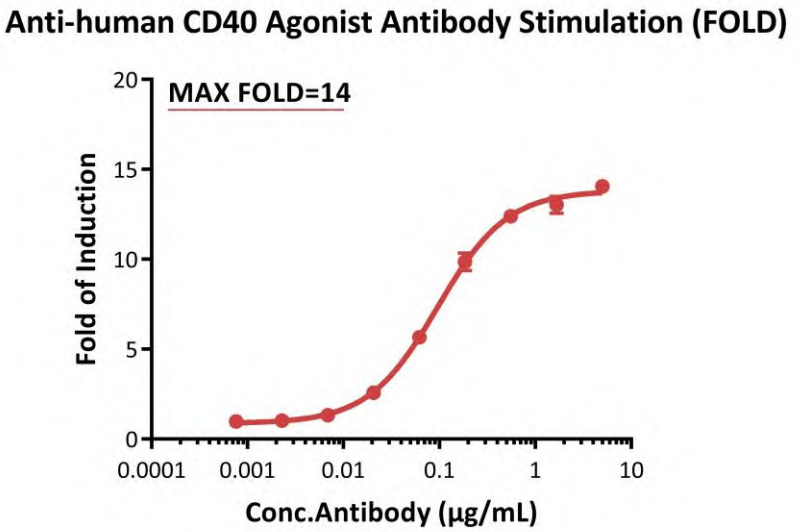

Fig3. Response to anti-human CD40 antibody (FOLD). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human CD40 antibody. The max induction fold was approximately 14.

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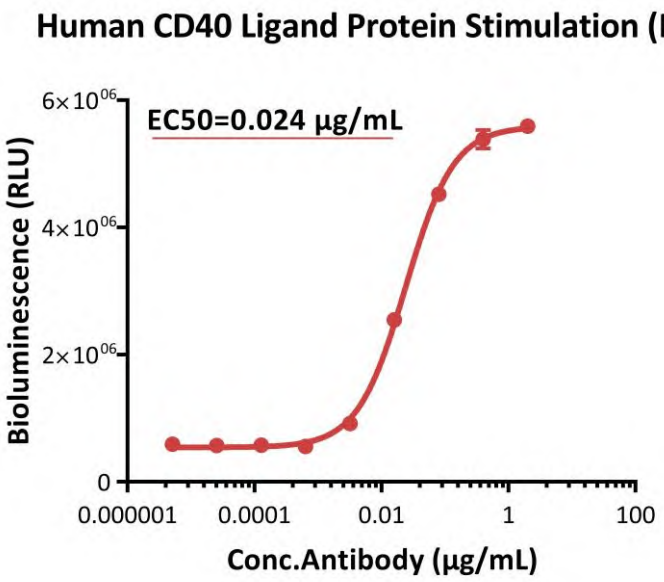

Fig4. Response to human CD40 ligand protein (RLU). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human CD40 ligand protein. The EC50 was approximately 0.024 μg/mL.

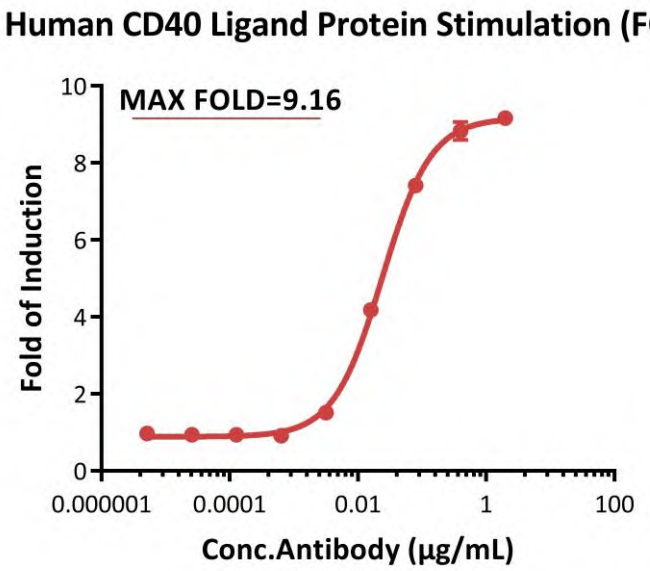

Fig5. Response to human CD40 ligand protein (FOLD). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human CD40 ligand protein. The max induction fold was approximately 9.16.

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- ***Application***

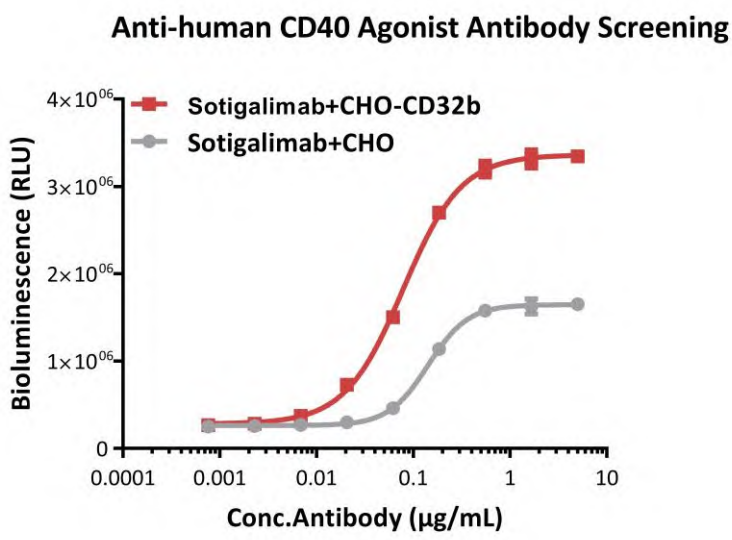

Fig6. Agonistic activity analysis of anti-human CD40 antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of CHO or CHO/CD32b. Sotigalimab could depend on CD32b-mediated crosslinking to strengthen CD40 signaling. The EC50 of Sotigalimab in the presence of CHO/CD32b was approximately 0.079 μg/mL.

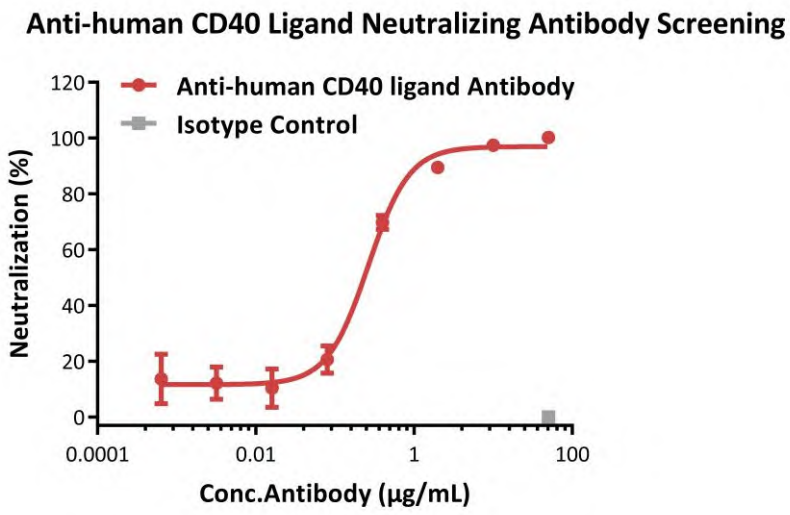

Fig7. Inhibition of human CD40 ligand protein-induced reporter activity by anti-human CD40 ligand neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human CD40 ligand protein with a final concentration of 0.1 μg/mL. The EC50 of anti-human CD40 ligand neutralizing antibody (Frexalimab) was approximately 0.26 μg/mL.

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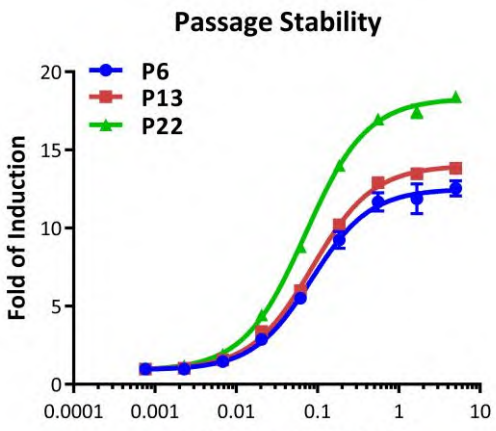
• *Passage Stability*



	P6	P13	P22
EC50(μg/mL)	0.084	0.086	0.071
Max Fold	13	14	18

Fig8. Passage stability analysis by Signaling Bioassay. The continuously growing Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human CD40 antibody. Anti-human CD40 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 6-22.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human CD40 (Luc) HEK293 Reporter Cell	CHEK-ATF097
Human 4-1BB (Luc) HEK293 Reporter Cell	CHEK-ATF073
CHO/Human CD32b Stable Cell Line (Low Expression)	CCHO-ATP060L
CHO/Human CD32b Stable Cell Line (Medium Expression)	CCHO-ATP060M
CHO/Human CD32b Stable Cell Line (High Expression)	CCHO-ATP060H