Human CD40 (Luc) HEK293 Reporter Cell Data Sheet

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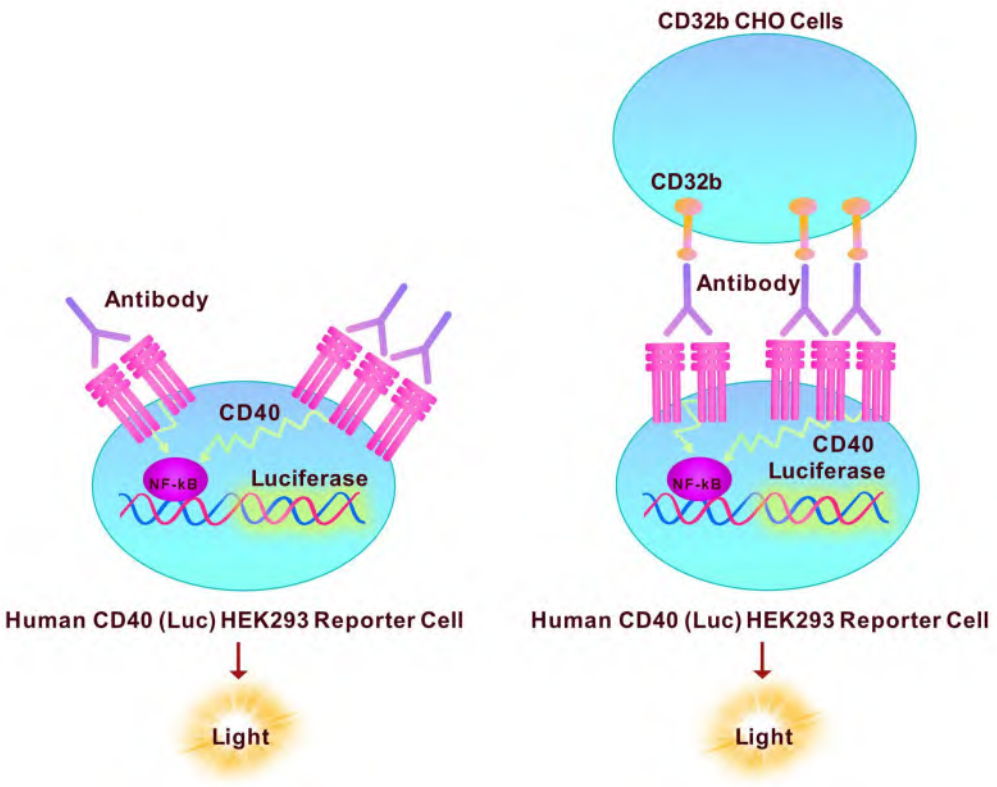
Catalog No.	Size
CHEK-ATF097	2 × (1 vial contains ~5×10^6 cells)

• *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 (Uniprot: P25942-1), 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 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),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)

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• *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℃ 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℃ 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.

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• *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 5×10^6 to 1×10^7 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.

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- ***Receptor Assay***

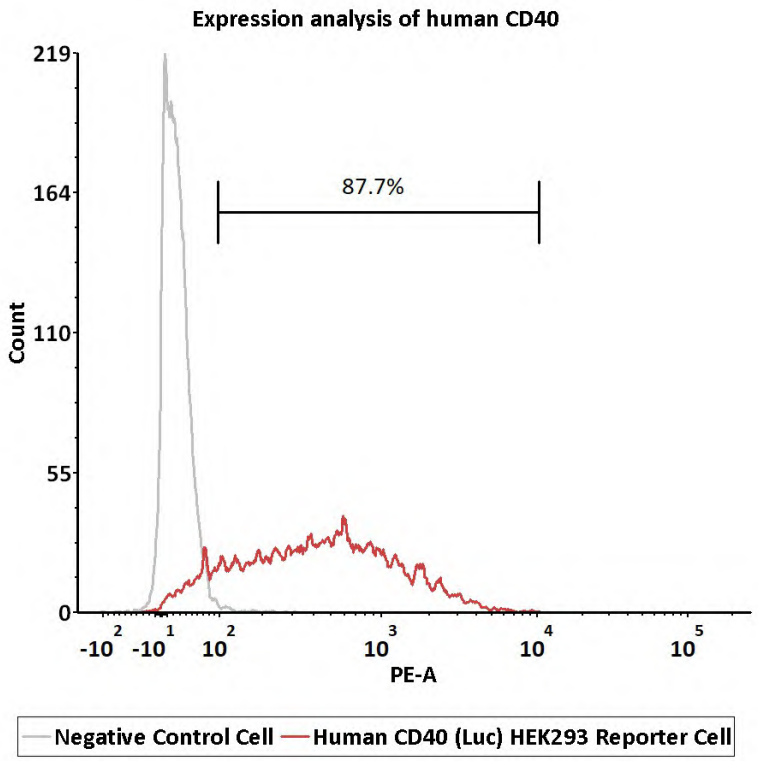

Fig1. Expression analysis of human CD40 on Human CD40 (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human CD40 (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human CD40 antibody.

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• *Signaling Bioassay*

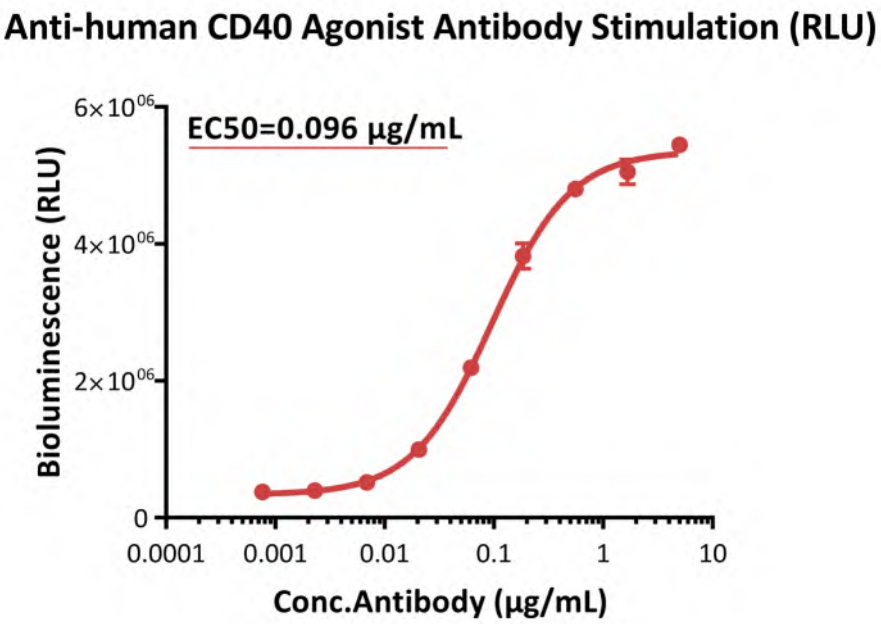

Fig2. Response to anti-human CD40 antibody (RLU). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human CD40 antibody. The EC50 was approximately 0.096 μg/mL.

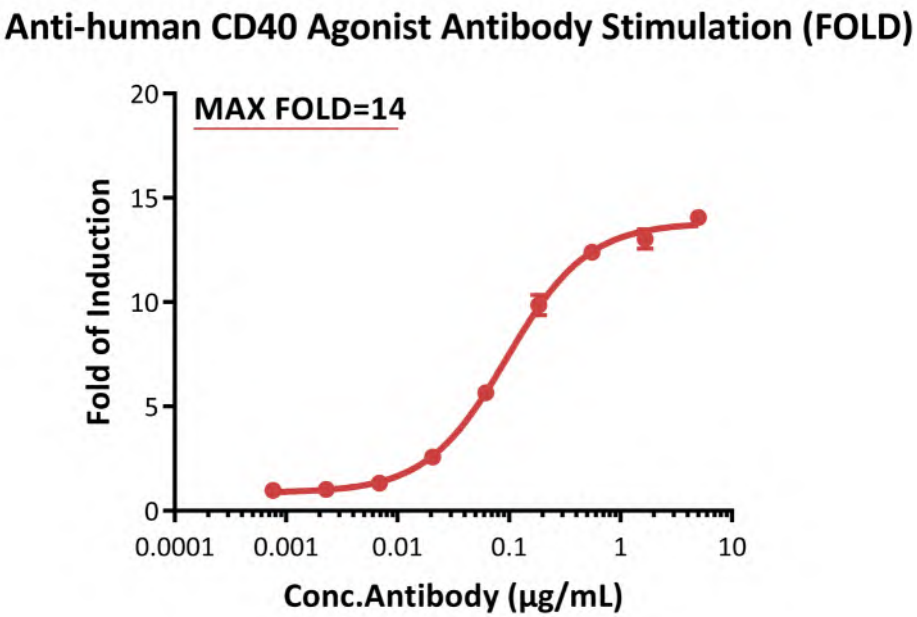

Fig3. Response to anti-human CD40 antibody (FOLD). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human CD40 antibody. The max induction fold was approximately 14.

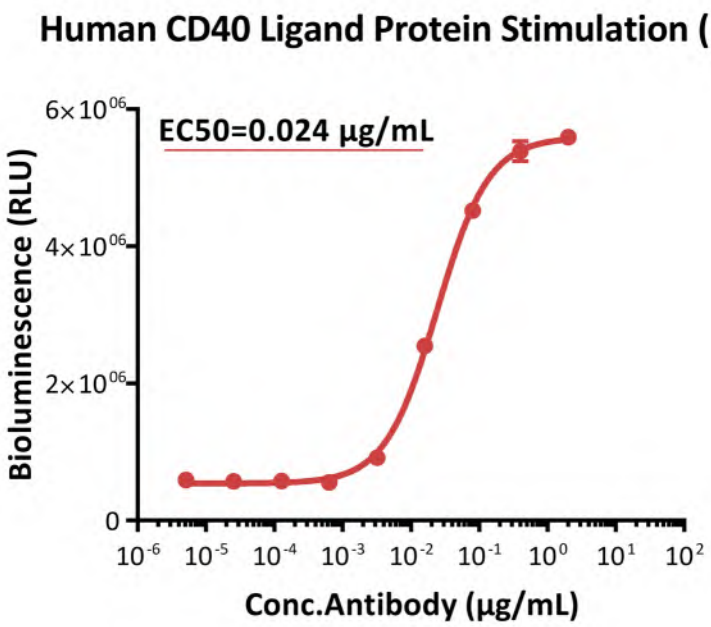

Fig4. Response to human CD40 ligand protein (RLU). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human CD40 ligand protein. The EC50 was approximately 0.024 μg/mL.

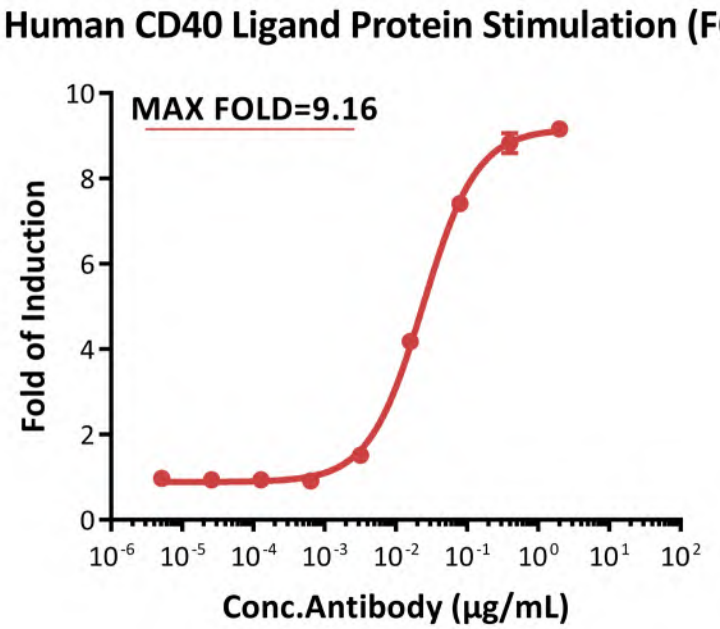

Fig5. Response to human CD40 ligand protein (FOLD). The Human CD40 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human CD40 ligand protein. The max induction fold was approximately 9.16.

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• ***Application***

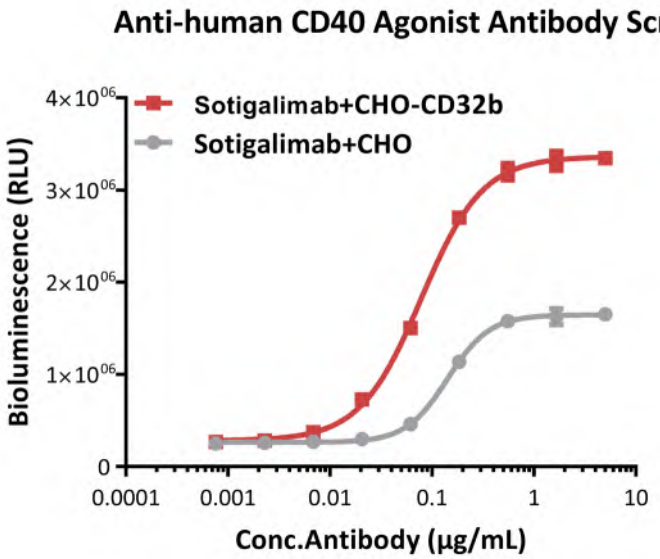


Fig6. Agonistic activity analysis of anti-human CD40 antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of CHO or CHO/CD32b. Sotigalimab could depend on CD32b-mediated crosslinking to strengthen CD40 signaling. The EC50 of Sotigalimab in the presence of CHO/CD32b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

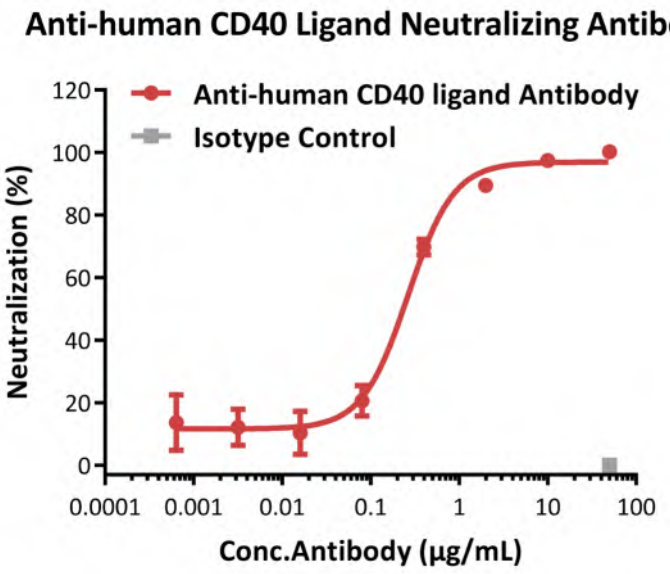
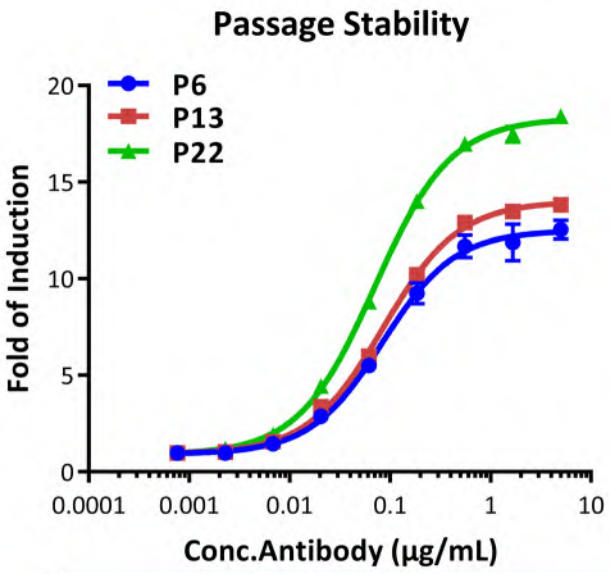


Fig7. Inhibition of human CD40 ligand protein-induced reporter activity by anti-human CD40 ligand neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human CD40 ligand protein with a final concentration of 0.1 μg/mL. The EC50 of anti-human CD40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

• ***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.

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• *Related Products*

Products	Cat.No.
Human TSLP R (Luc) HEK293 Reporter Cell	CHEK-ATF045
STAT3 (Luc) HEK293 Reporter Cell	CHEK-ATF047
HEK293/Human CD40 Ligand / TNFSF5 Stable Cell Line	CHEK-ATP041
HEK293/Human OX40 / TNFRSF4 / CD134 Stable Cell Line	CHEK-ATP053
HEK293/Human OX40 Ligand / TNFSF4 Stable Cell Line	CHEK-ATP054
Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell	CHEK-ATF074
Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell	CHEK-ATF075
HEK293/FcRn (FCGRT & B2M) Cell Line	CHEK-ATP079
Human IL-21 R (Luc) HEK293 Reporter Cell	CHEK-ATF051
Human IL-11 R alpha (Luc) HEK293 Reporter Cell	CHEK-ATF052
CHO/Human TSHR Stable Cell Line	SCCHO-ATP085
HEK293/Human TSHR Stable Cell Line	CHEK-ATP086
Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell	CHEK-ATF094
Human IL-10 R alpha/IL-10 R beta (Luc) HEK293 Reporter Cell	CHEK-ATF095
Human IL-7 R alpha/CD132 (Luc) HEK293 Reporter Cell	CHEK-ATF099
NIH-3T3/Human IGF-1 R Stable Cell Line	CNIH-ATP102
Human HVEM (Luc) HEK293 Reporter Cell	CHEK-ATF105
Human BTLA (Luc) Jurkat Reporter Cell	SCJUR-STF106
Human IGF-1 R (Luc) HEK293 Reporter Cell	CHEK-ATF107
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
Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell	CHEK-ATF133
Human OX40 (Luc) HEK293 Reporter Cell	CHEK-ATF135
Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell	CHEK-ATF136
HEK293/Human TL1A Stable Cell Line	CHEK-ATP142
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
Human DR3 (TL1A receptor) (Luc) Jurkat Reporter Cell	SCJUR-STF178

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• *Related Products*

Products	Cat.No.
CHO/Human LILRB4 Stable Cell Line	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 OX40 (Luc) HEK293 Reporter Cell	CHEK-ATF135
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