

Human 4-1BB (Luc) HEK293 Reporter Cell Data Sheet

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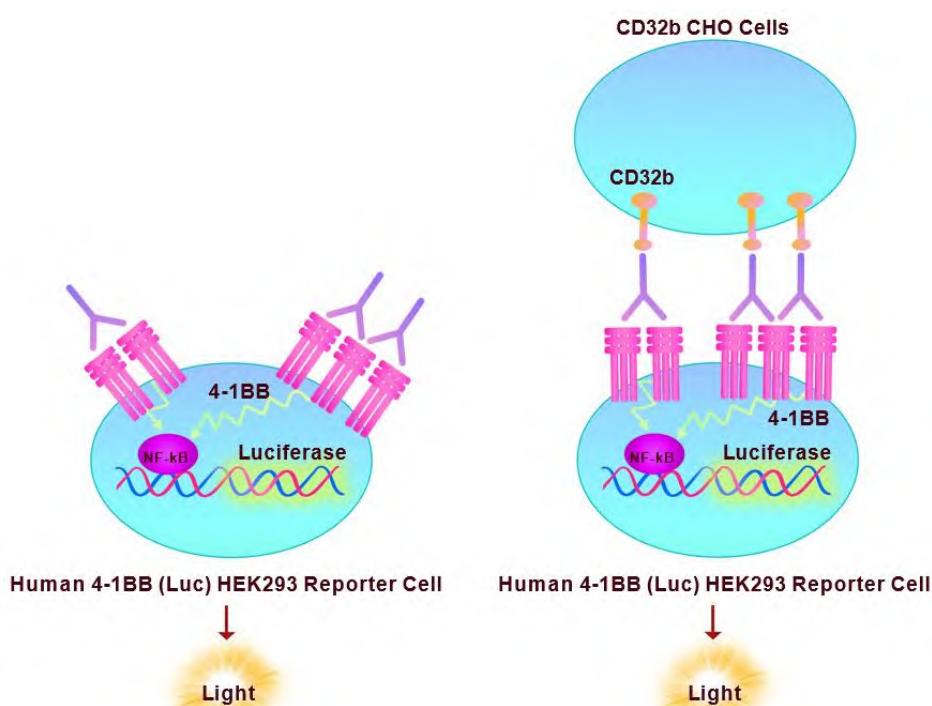
Catalog No.	Size
CHEK-ATF073	2 × (1 vial contains ~5×10 ⁶ cells)

• Description

The Human 4-1BB (Luc) HEK293 Reporter Cell was engineered to not only express NF- κ B signaling response element, but also express the receptor full length human 4-1BB (Uniprot: Q07011), which can drive luciferase expressing systems by 4-1BB ligand/ agonist antibody stimulation. In the absence of agonist antibody or 4-1BB ligand, the 4-1BB receptor is not activated and luminescence signal is low. In the presence of agonist antibody or 4-1BB ligand, the 4-1BB 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 Fc γ R-dependent manner to strengthen the agonistic activity.

• Application

- Screen for ligands or agonist antibodies that can bind and activate 4-1BB



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• Cell Line Profile

Cell line	Human 4-1BB (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°C 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)
- CO₂ 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°C with 5% CO₂ 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% confluence. 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% CO₂ incubator.
7. When the cell culture reaches 80-90% confluence, proceed to the next subculture. Avoid over-confluence, 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% confluence, 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

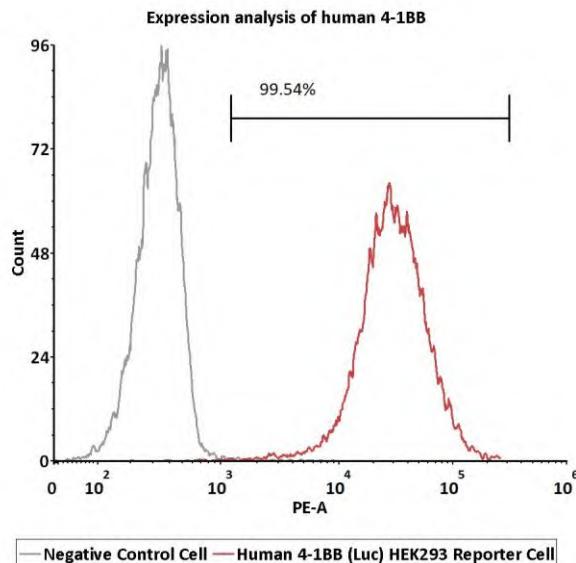


Fig1. Expression analysis of human 4-1BB on Human 4-1BB (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human 4-1BB (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human 4-1BB antibody.

• Signaling Bioassay

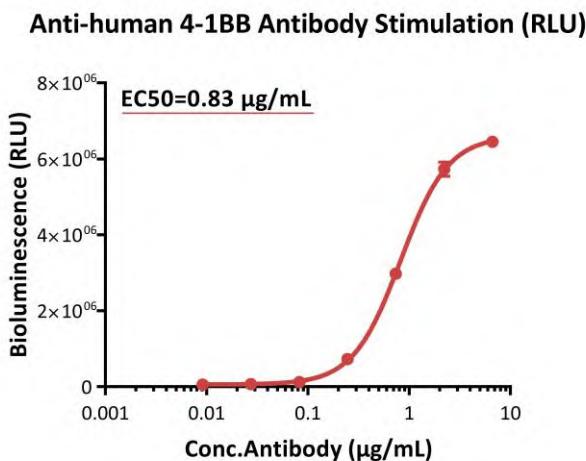


Fig2. Response to Anti-human 4-1BB antibody (RLU). The Human 4-1BB (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human 4-1BB antibody. The EC50 was approximately 0.83 μg/mL.

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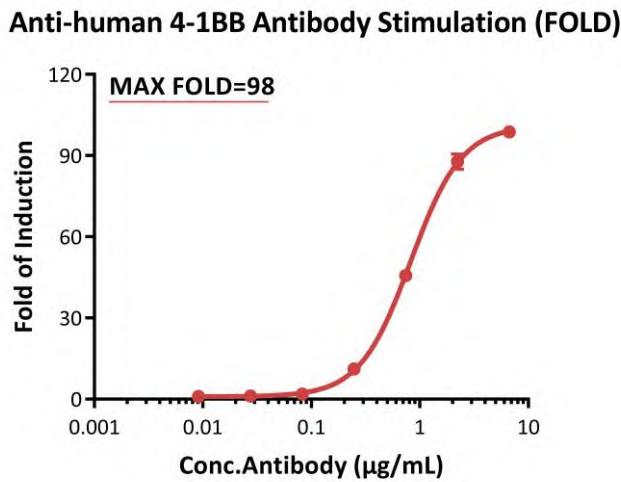


Fig3. Response to Anti-human 4-1BB antibody (FOLD). The Human 4-1BB (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Anti-human 4-1BB antibody. The max induction fold was approximately 98.

• Application

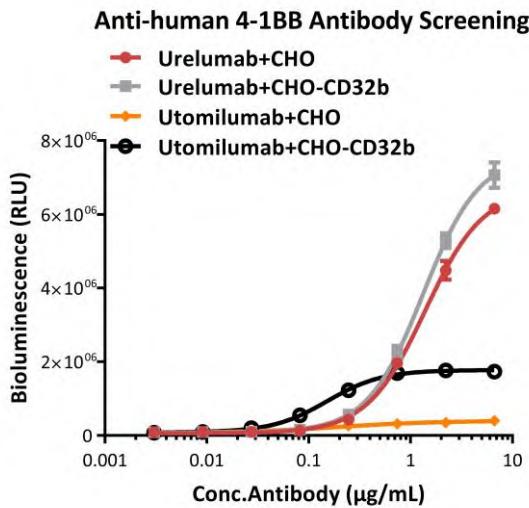
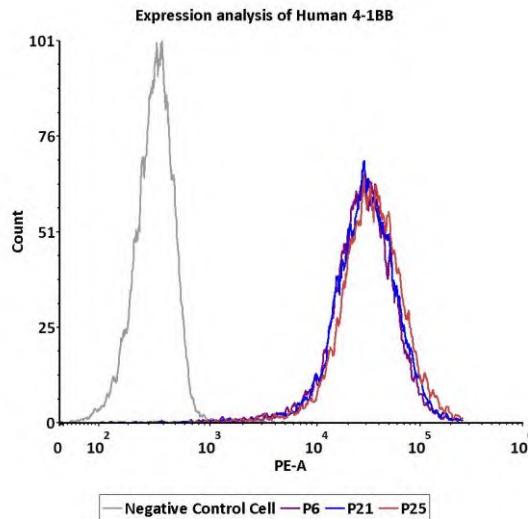


Fig4. Agonistic activity analysis of anti-human 4-1BB antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of CHO or CHO/CD32b. Urelumab, a strong intrinsic agonistic antibody, can activate 4-1BB signaling independent of CD32b-mediated crosslinking. Utomilumab, a weak agonistic antibody, can activate 4-1BB signaling dependent on CD32b-mediated crosslinking.

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• Passage Stability



Passage	MFI for human 4-1BB (PE)
P6	27878.53
P21	29033.39
P25	32847.21

Fig5. Passage stability analysis of human 4-1BB expression by FACS. Flow cytometry surface staining of human 4-1BB on Human 4-1BB (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 6-25.

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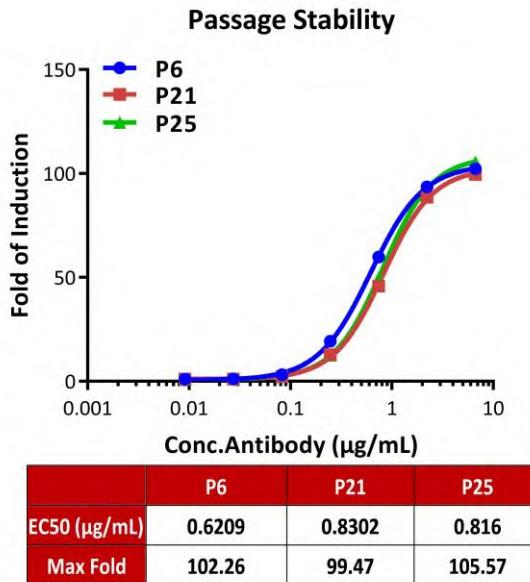


Fig6. Passage stability analysis by Signaling Bioassay. The continuously growing Human 4-1BB (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Anti-human 4-1BB antibody. Anti-human 4-1BB antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 6-25.

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• *Related Products*

<u>Products</u>	<u>Cat.No.</u>
CHO/Human CD16a (158V) Stable Cell Line (Low Expression)	SCCHO-ATP059L
CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)	SCCHO-ATP059M
CHO/Human CD16a (158V) Stable Cell Line (High Expression)	SCCHO-ATP059H
CHO/Human CD32b Stable Cell Line (Low Expression)	SCCHO-ATP060L
CHO/Human CD32b Stable Cell Line (Medium Expression)	SCCHO-ATP060M
CHO/Human CD32b Stable Cell Line (High Expression)	SCCHO-ATP060H
CHO/Human CD32a Stable Cell Line (Low Expression)	SCCHO-ATP061L
CHO/Human CD32a Stable Cell Line (Medium Expression)	SCCHO-ATP061M
CHO/Human CD32a Stable Cell Line (High Expression)	SCCHO-ATP061H
CHO/Human CD64 Stable Cell Line (Low Expression)	SCCHO-ATP062L
CHO/Human CD64 Stable Cell Line (Medium Expression)	SCCHO-ATP062M
CHO/Human CD64 Stable Cell Line (High Expression)	SCCHO-ATP062H
CHO/Human PD-L1 Stable Cell Line (Low Expression)	SCCHO-ATP077L
CHO/Human PD-L1 Stable Cell Line (Medium Expression)	SCCHO-ATP077M
CHO/Human PD-L1 Stable Cell Line (High Expression)	SCCHO-ATP077H
HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line	CHEK-ATP039
HEK293/Human 4-1BB / TNFRSF9 Stable Cell Line	CHEK-ATP038