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

Human 4-1BB (Luc) HEK293 Reporter Cell

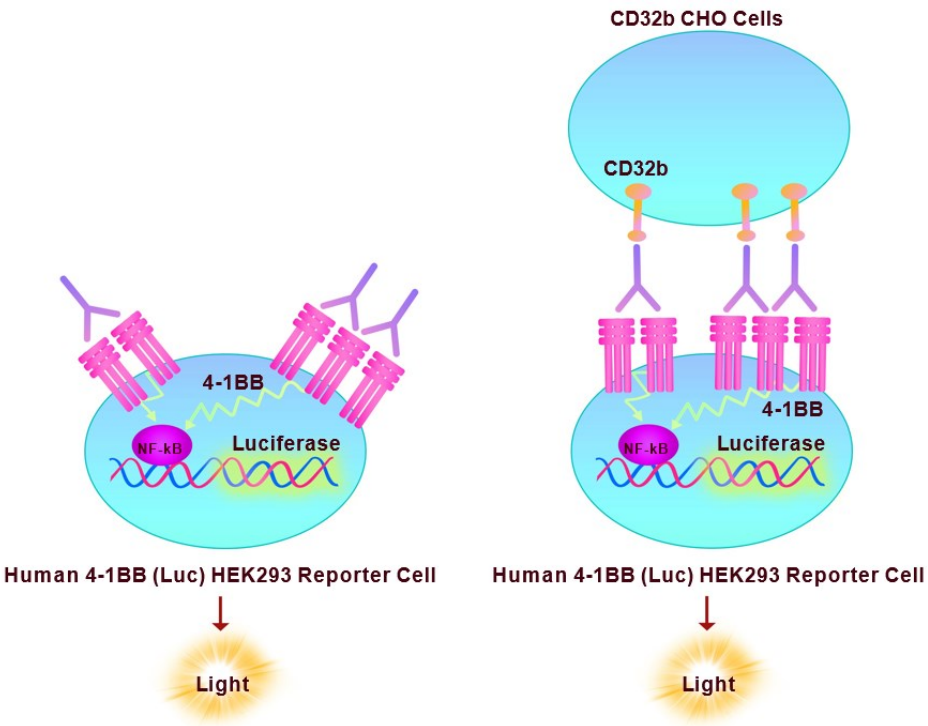
Catalog No.	Size
CHEK-ATF073	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human 4-1BB (Luc) HEK293 Reporter Cell was engineered to not only express NF-κB signaling response element, but also express the receptor full length human 4-1BB（Gene ID: 3604), 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 FcγR-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 (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 (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, Hygromycin (20 μg/mL), Puromycin (2 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 3 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 5-7 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***

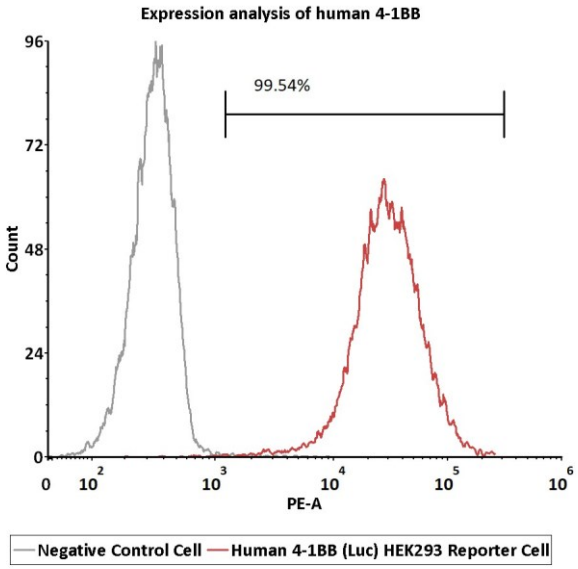

Fig1. Expression analysis of human 4-1BB on Human 4-1BB (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human 4-1BB (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human 4-1BB antibody.

- ***Signaling Bioassay***

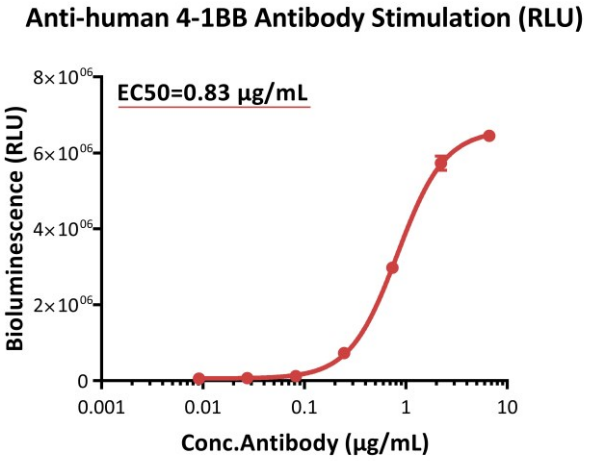

Fig2. Response to Anti-human 4-1BB antibody (RLU). The Human 4-1BB (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of anti-human 4-1BB antibody. The EC50 was approximately 0.83 μg/mL.

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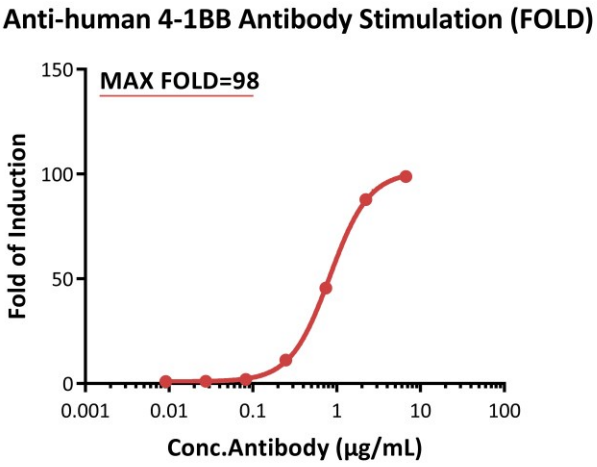

Fig3. Response to Anti-human 4-1BB antibody (FOLD). The Human 4-1BB (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Anti-human 4-1BB antibody. The max induction fold was approximately 98.

- ***Application***

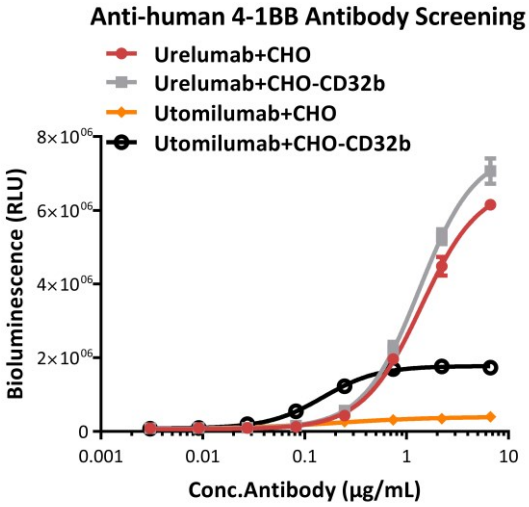

Fig4. Agonistic activity analysis of anti-human 4-1BB antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of CHO or CHO/CD32b. Urelumab, a strong intrinsic agonistic antibody, can activate 4-1BB signaling independent of CD32b-mediated crosslinking. Utomilumab,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

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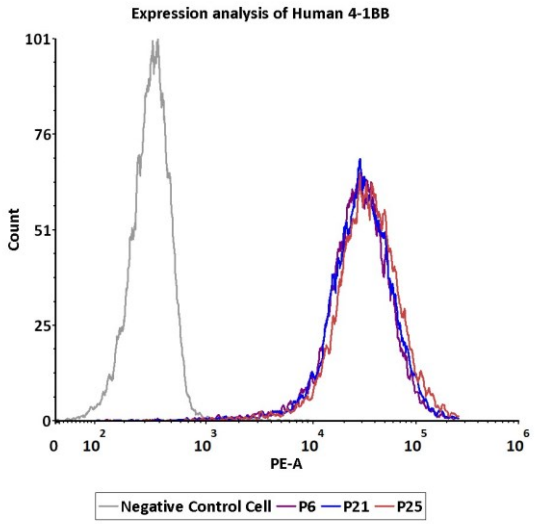
- ***Passage Stability***



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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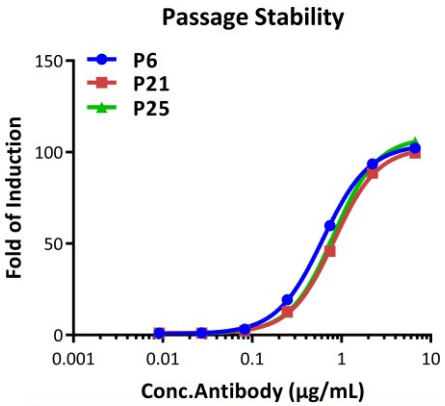

	P6	P21	P25
EC50(ug/mL)	0.6209	0.8302	0.816
Max Fold	102.26	99.47	105.57

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.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
CHO/Human CD16a (158V) Stable Cell Line (Low Expression)	CCHO-ATP059L
CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)	CCHO-ATP059M
CHO/Human CD16a (158V) Stable Cell Line (High Expression)	CCHO-ATP059H
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
CHO/Human CD32a Stable Cell Line (Low Expression)	CCHO-ATP061L
CHO/Human CD32a Stable Cell Line (Medium Expression)	CCHO-ATP061M
CHO/Human CD32a Stable Cell Line (High Expression)	CCHO-ATP061H
CHO/Human CD64 Stable Cell Line (Low Expression)	CCHO-ATP062L
CHO/Human CD64 Stable Cell Line (Medium Expression)	CCHO-ATP062M
CHO/Human CD64 Stable Cell Line (High Expression)	CCHO-ATP062H
CHO/Human PD-L1 Stable Cell Line (Low Expression)	CCHO-ATP077L
CHO/Human PD-L1 Stable Cell Line (Medium Expression)	CCHO-ATP077M
CHO/Human PD-L1 Stable Cell Line (High Expression)	CCHO-ATP077H