

## HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line Data Sheet

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## HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line

Catalog No.	Size
CHEK-ATP039	2 × (1 vial contains ~5×10 <sup>6</sup> cells)

### • *Description*

The HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line was engineered to express the full length human 4-1BB Ligand / TNFSF9 (Uniprot: P41273-1). Surface expression of human 4-1BB Ligand / TNFSF9 was confirmed by flow cytometry.

### • *Application*

- Useful for cell-based 4-1BB Ligand / TNFSF9 binding assay.

### • *Cell Line Profile*

Cell line	HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (5 µg/mL)
Incubation	37°C with 5% CO <sub>2</sub>
Doubling Time	22-24 hours
Transduction Technique	Plasmid

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## • *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)

**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 (5 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<sub>2</sub> 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% CO<sub>2</sub> 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<sub>2</sub> 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  $5 \times 10^6$  to  $1 \times 10^7$  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.

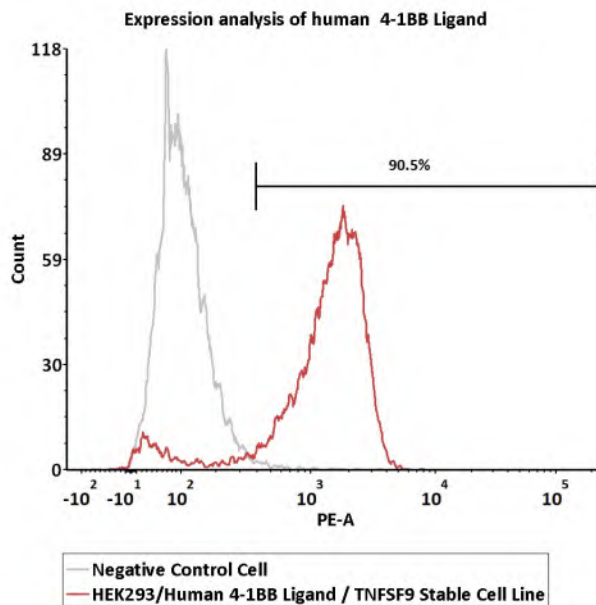
**Note:** It is recommended to establish a cell bank at the earliest possible passage for long-term use.

## • *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



Catalog No.	Stable Cell Line	MFI for 4-1BB Ligand / TNFSF9 (PE)
NA	Negative Control Cell	92.74
CHEK-ATP039	HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line	1447.23

**Fig 1. Expression analysis of human 4-1BB Ligand / TNFSF9 on HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line by FACS.** Cell surface staining was performed on HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line or negative control cell using biotinylated human 4-1BB / TNFRSF9 protein (Cat. No. 41B-H82F8) followed by staining with Streptavidin Protein-PE (Cat. No. STN-NP119).

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### • *Related Products*

#### Products

#### Cat. No.

HEK293/Human PD-L1, GFP Tag Stable Cell Line	CHEK-ATP002
HEK293/Human 4-1BB / TNFRSF9 Stable Cell Line	CHEK-ATP038
Human PD-1/LAG-3 (Luc) Jurkat Reporter	SCJUR-STF063
Human PD-1 (Luc) Jurkat Reporter Cell	SCJUR-STF064
Human LAG-3 (Luc) Jurkat Reporter Cell	SCJUR-STF065
Raji/Human PD-L1 Stable Cell Line	SCRAJ-STT075
Raji/Human CD155 Stable Cell Line	SCRAJ-STT076
CHO/Human LILRB4 Stable Cell Line	SCCHO-ATP087
HEK293/Human LILRB4 Stable Cell Line	CHEK-ATP088
Raji/Human HVEM Stable Cell Line	SCRAJ-STF108
CHO/Human LIGHT Stable Cell Line	SCCHO-ATP109
CHO/Human BTLA Stable Cell Line	SCCHO-ATP110
HEK293/Human PD-1 Stable Cell Line	CHEK-ATP143
HEK293/Human HVEM Stable Cell Line	CHEK-ATP147
HEK293/Human NKp46 Stable Cell Line	CHEK-ATP153
HEK293/Human ITPRIPL1 Stable Cell Line	CHEK-ATP203
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130