

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

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

Catalog No.	Clone No.	Size
CHEK-ATP039	AC922C1	1 vial containing at least 5x10 <sup>6</sup> cells

### • Description

HEK293/Human 4-1BB Ligand Stable Cell Line.

### • Cell Line Profile

Cell line	HEK293/Human 4-1BB Ligand Stable Cell Line
Species	Human
Property	Adherent
Medium	DMEM medium +10% FBS
Selection Marker	Puromycin (5 µg/mL)
Incubation	37°C with 5% CO <sub>2</sub>
Storage	Frozen in liquid nitrogen
Biosafety Level	1
Application	Binding assay by FACS and cell based ELISA.

### • *Materials Required for Cell Cultur*

- DMEM Culture Medium (BasalMedia, Cat.No. L120KJ)
- Fetal bovine serum (CellMax, Cat.No.SA211.01)
- Trypsin (Gibco, Cat.No. 15050065)
- PBS (CellMax, Cat.No.CBS101.05)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- DMSO (Applichem, Cat.No. A3672,0250)
- 90mm-culture dishes (SARSTEDT, Cat.No.83-3902)
- Cryogenic storage vials (greiner, Cat.No.122280)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Cellaca, MX)
- CO<sub>2</sub> Incubator (Thermo, 371)
- Biological Safety Cabinet (HDL, BSC-1360IIA2)

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## • **Recovery**

- a. Rapidly thaw (< 2 minute) frozen vial of cell in a 37°C-water bath.
- b. Transfer the cell suspension into a tube with 5 mL complete culture medium. Complete culture medium contains 90% DMEM and 10% FBS.
- c. Spin down the cells at  $110 \times g$  for 5 minutes.
- d. Resuspend cell pellet with appropriate volume of complete culture medium and transfer the cell suspension into two 90mm-culture dishes.
- e. Incubate at 37 °C with 5% CO<sub>2</sub> incubator until the cells are ready to be split.

## • **Subculture**

- a. Viability may be poor on resuscitation, full recovery may take up to a week. Observe continuously every day until the cell confluency reaches 90%, remove and discard spent medium.
  - b. Wash the cells once with sterile PBS.
  - c. Add 3 mL of trypsin to cell culture dish. Observe the cells under microscope until 90% of the cells have detached.
  - d. Add 5~7 mL complete medium to neutralize trypsin.
  - e. Spin down the cells at  $110 \times g$  for 5 minutes.
  - f. Discard the supernatants and add 3~5mL of complete medium and aspirate cells by gently pipetting. Split cells 1:3 to 1:5.
  - g. Incubate at 37 °C with 5% CO<sub>2</sub> incubator.
  - h. 3 days later, cell confluency can reach 90%.
- Note: Add 5 µg/mL Puromycin from first subculture.

## • **Cryopreservation**

- a. The best freezing time is the second week after resuscitation. Freeze the cells at a final density between  $5 \times 10^6$  and  $2 \times 10^7$  viable cells/mL.
  - b. Use a freezing medium composed of 90% FBS and 10% DMSO.
- Note: Check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen by following the procedure outlined in Recovery.