HEK293/Human OX40 Stable Cell Line Data Sheet

HEK293/Human OX40 Stable Cell Line

Catalog No.	Clone No.	Size
CHEK-ATP053	AC1096C1	1 vial containing at least 5x10^6 cells

• Description

HEK293/Human OX40 Stable Cell Line.

• Cell Line Profile

Cell line	HEK293/Human OX40 Stable Cell Line
Species	Human
Property	Adherent
Medium	DMEM medium +10% FBS
Selection Marker	Puromycin (5 μg/mL)
Incubation	37℃ with 5% CO_2
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)
- Recombinant Trypsin-EDTA Solution (Beyotime, Cat.No. C0208-100mL)
- 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_2 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℃-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 × 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 (try to avoid using T-Flask).

e. Incubate at 37 ℃ with 5% CO_2 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, the digestion time should be controlled within 10 minutes (contact us when your Trypsin is other brands).

d. Add 5~7 mL complete medium to neutralize trypsin.

e. Spin down the cells at 110 × 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 ℃ with 5% CO_2 incubator.

h. 3-4 days later, cell confluency can reach 90%.

*Note: Add **5 μg/mL Puromycin** from second subculture, and make sure the cell viability should be ⩾90%.*

• *Cryopreservation*

a. The best freezing time is the second week after resuscitation. Freeze the cells at a final density between 5 × 10^6 and 2 × 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.