

HEK293/Human Mesothelin Stable Cell Line

Catalog No.	Size
CHEK-ATP119	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

• Description

The HEK293/Human Mesothelin Stable Cell Line was engineered to express the receptor full length human Mesothelin (Gene ID: 10232), used to mimic cancer target cells. Surface expression of human Mesothelin was confirmed by flow cytometry.

• Application

Useful for cell-based Mesothelin binding assay

• Cell Line Profile

Cell line	HEK293/Human Mesothelin Stable Cell Line
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 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)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, 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)
- CO₂ Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)



• 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° C with 5% CO₂ 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 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 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 $^\circ\!\mathrm{C}$ 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.

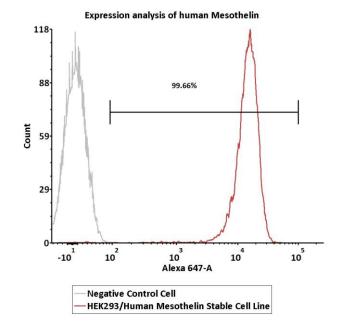


• Cryopreservation

- 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 complete growth 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 $a 80^{\circ}$ C freezer overnight, then transferring to liquid nitrogen storage.
- Storage
 - **Product format:** Frozen
 - Storage conditions: Liquid nitrogen immediately upon receipt



• Receptor Assay



Catalog No.	Stable Cell Line	MFI for Mesothelin (Alexa 647)
NA	Negative Control Cell	4.33
CHEK-ATP119	HEK293/Human Mesothelin Stable Cell Line	14439.38

Fig1. Expression analysis of human Mesothelin on HEK293/Human Mesothelin Stable Cell Line by FACS.

Cell surface staining was performed on HEK293/Human Mesothelin Stable Cell Line or negative control cell using Alexa 647-labeled anti-human Mesothelin antibody.



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• Related Products

Products	<u>Cat.No.</u>
HEK293/Human CEACAM5 Stable Cell Line	CHEK-ATP083
HEK293/Human ROR1 Stable Cell Line	CHEK-ATP084
HEK293/Human Glypican-3 (GPC3) Stable Cell Line	CHEK-ATP092
HEK293/Human FOLR1 Stable Cell Line	CHEK-ATP091