

HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line

Catalog No.	Size
CHEK-ATP199	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

• Description

The HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line was engineered to not only express the receptor full length human IDH1 mutated to a Histidine (H) at amino acid 132 and mGFP by a viral P2A self-cleaving peptide, but also express the firefly luciferase reporter. The expression of mGFP characterizing the human IDH1 (132H) was confirmed by flow cytometry. The Luciferase activity was confirmed by the detection of luminescence signal.

• Application

• Screen for human IDH1 inhibitor

• Cell Line Profile

Cell line	HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	
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)
- 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 (2 µg/mL), Hygromycin (20 µg/mL), 1%P/S
- 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°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.

Note: After recovery for 1-2 generations with the complete growth medium not containing the selection marker, if the cell state is well, changing to the culture medium containing the selection marker.



• 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 IDH1 (132H) (FITC)
NA	Negative Control Cell	12.31
CHEK-ATP199	HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	25412.03

Fig1. Expression analysis of human IDH1 (132H) on HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line by FACS. The expression of mGFP characterizing the human IDH1 (132H) was detected by flow cytometry.



• Application



Fig2. Expression analysis of Luciferase on HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line (RLU). The luminescence signal of HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line were detected by incubating with the luciferase substrate. The RLU value was approximately 5.84×10^{10} at the density of 1×10^{10} cells/well.

Luciferase activity detection





Fig3. Luciferase activity of HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line (FOLD). The luminescence signal of HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line were detected by incubating with the luciferase substrate. The induction fold was approximately 2171.46.



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• Related Products

Products	<u>Cat.No.</u>
CHO/Human Mesothelin Stable Cell Line Development Service	SCCHO-ATP120
CHO/Human Glypican-3 (GPC3) Stable Line Development Service	SCCHO-ATP112
CHO/Human STEAP1 Stable Cell Line Development Service	SCCHO-ATP121
CHO/Human uPAR Stable Cell Line Development Service	SCCHO-ATP152
CHO/Human c-MET Stable Cell Line Development Service	SCCHO-ATP141
HEK293/Human ROR1 Stable Cell Line	CHEK-ATP084
HEK293/Human Mesothelin Stable Cell Line	CHEK-ATP119
HEK293/Human Glypican-3 (GPC3) Stable Cell Line	CHEK-ATP092
HEK293/Human FOLR1 Stable Cell Line	CHEK-ATP091
HEK293/Human DLL3 Stable Cell Line	CHEK-ATP090
HEK293/Human TL1A Stable Cell Line	CHEK-ATP142
HEK293/Human NAPI-IIb Stable Cell Line	CHEK-ATP116
HEK293/Human Cadherin-6 Stable Cell Line	CHEK-ATP127
HEK293/Human ENPP3 Stable Cell Line	CHEK-ATP122
HEK293/Human B7-H4 Stable Cell Line	CHEK-ATP126
HEK293/Human Cadherin-17 Stable Cell Line	CHEK-ATP173
HEK293/Human IDH1(132R)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP200
MDCK/Mouse FCGRT-P2A-mGFP&B2M Cell Line Development Service	SCMDC-ATP196