

HEK293/Human Alpha-synuclein (GFP) Stable Cell Line Data Sheet

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Catalog No.	Clone No.	Size
CHEK-ATP085	AC1655P1	1 vial containing at least 5x10 ⁶ cells

• Description

HEK293/Human Alpha-synuclein (GFP) Stable Cell Line.

• Cell Line Profile

Cell line	HEK293/Human Alpha-synuclein (GFP) Stable Cell Line
Species	Human
Property	Adherent
Medium	DMEM medium +10% FBS
Selection Marker	Hygromycin B (250 µg/ml)
Incubation	37 °C with 5% CO ₂
Storage	Frozen in liquid nitrogen
Biosafety Level	1
Application	α-Synuclein PFF induced intracellular aggregation assay.

• *Materials Required for Cell Culture*

- 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)
- Hygromycin B (InvivoGen, Cat.No.ant-hg-1)
- 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)
- CO₂ 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% CO₂ 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% CO₂ incubator.
- h. 3 days later, cell confluency can reach 90%.

Note: Add 250 µg/mL Hygromycin B from first subculture.

• **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.