

HK EB3-EGFP Cells | 300668

General information

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| Description | HeLa Kyoto EB3-EGFP cells carry specific markers known as EB3-EGFP, enabling the visualization and study of microtubules, the dynamic structures essential for cellular processes. This clonal stable cell line was generated by transfection of a circular plasmid followed by drug resistance selection. |
| Organism | Human |
| Tissue | Cervix |
| Disease | Carcinoma |
| Synonyms | HeLa Kyoto EB3-EGFP, HeLa Kyoto EB3 EGFP, HeLa Kyoto EGFP-EB3 |

Characteristics

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|--------------------------|---|
| Age | 30 years |
| Gender | Female |
| Ethnicity | African American |
| Morphology | Epithelial-like cells with mosaic stone shape |
| Growth properties | Monolayer, adherent |

Identifiers / Biosafety / Citation

| | |
|------------------------|--|
| Citation | HK EB3-EGFP (Cytion catalog number 300668) |
| Biosafety level | 1 |
| Depositor | Dr. J. Ellenberg, EMBL Heidelberg |

Expression / Mutation

| | |
|---------------------------|--|
| Protein expression | mEGFP (microtubule End-binding protein 3 mEGFP tagged): Location/Gene: 1..589 / Pcmv, 652..1497 / EB3, 1516..2235 / EGFP, 3466..4260 / KanR/NeoR |
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Products CMV Promotor EB3, Neomycin, Phosphotransferase

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Handling

Culture Medium DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO₃, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)

Medium supplements Supplement the medium with 10% FBS

Passaging solution Accutase

Subculturing Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

Split ratio A ratio of 1:3 is recommended

Seeding density 1×10^4 cells/cm²

Fluid renewal 2 to 3 times per week

Freezing recovery After thawing, plate the cells at 5×10^4 cells/cm² and allow the cells to recover from the freezing process and to adhere for at least 24 hours.

Freeze medium CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

Handling of cryopreserved cultures HK EB3-EGFP cells are shipped in a deep-frozen state on dry ice. Upon receipt, confirm that the vial remains frozen. For storage, place the cryovial immediately at temperatures below -150 degrees. If you plan to culture the cells immediately, swiftly thaw the vial by shaking it in a 37 degrees water bath with clean water and an antimicrobial agent for 40-60 seconds. Remove the vial once a small ice clump persists, ensuring it remains cold. Proceed with all subsequent steps under aseptic conditions. In a sterile flow hood, disinfect the cryovial with 70% ethanol. Then, gently open the vial and transfer the cell suspension into a 15 ml centrifuge tube pre-filled with 8 ml of room temperature culture medium. Gently mix the cells. For cell separation, centrifuge at 300 x g for 3 minutes and dispose of the supernatant. Skipping centrifugation is optional, although any residual freezing medium should be removed after 24 hours. Resuspend the pellet gently in 10 ml of fresh culture medium and divide between two T25 culture flasks. Follow the subculture protocol for subsequent steps.

Quality control / Genetic profile / HLA

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Sterility

Mycoplasma contamination is rigorously excluded using both PCR-based assays and luminescence-based mycoplasma detection methods. To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.