Product sheet

HK EB3-EGFP Cells | 300668

Renseignements généraux

Description	Hela Kyoto EB3-EGFP is a derivative of the HeLa Kyoto cell line, specifically engineered to express the End-Binding Protein 3 (EB3) tagged with Enhanced Green Fluorescent Protein (EGFP). This cell line is commonly utilized in research focused on understanding microtubule dynamics due to the fluorescent tagging of EB3, a protein that associates with the plus ends of microtubules. The expression of EGFP provides a fluorescent marker that allows for real-time visualization of microtubule behavior in live cells under a fluorescence microscope. This cell line is particularly valuable in cell biology and cancer research, where understanding the mechanics of cell division and intracellular transport is crucial. The stable expression of EB3-EGFP does not interfere with the normal functions of the microtubules, making these cells a reliable tool for detailed studies of cellular processes that depend on microtubule dynamics.
Organism	Human
Tissue	Cervix
Disease	Carcinoma
Synonyms	HeLa Kyoto EB3-EGFP, HeLa Kyoto EB3 EGFP, HeLa Kyoto EGFP-EB3

Caractéristiques

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

Données réglementaires

Citation	HK EB3-EGFP (Cytion catalog number 300668)
Biosafety level	1

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NCBI_TaxID	9606
CellosaurusAccession	CVCL_1D61
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This HeLa Kyoto EB3-EGFP line contains an EGFP-tagged EB3 construct for dynamic microtubule visualization. This classification applies only within Germany and may differ elsewhere.

Données biomoléculaires

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
Products	CMV Promotor EB3, Neomycin, Phosphotransferase

Manipulation

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
Supplements	Supplement the medium with 10% FBS
Dissociation Reagent	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.
Seeding density	1 x 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week
Post-Thaw Recovery	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hours.

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Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.
Thawing and Culturing Cells	1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required. 3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains. 4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening. 5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently. 6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. 7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth. 8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.
Incubation Atmosphere	37°C, 5% CO_2, humidified atmosphere.
Shipping Conditions	Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.
Storage Conditions	For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Contrôle de la qualité et analyse moléculaire

Sterility

Mycoplasma contamination is 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.