

HK EGFP-Kleisin-beta Cells | 300674

Description

The HK EGFP-Kleisin-beta cell line represents a genetically modified variant of HeLa Kyoto cells designed primarily for the study of chromosome cohesion during the cell cycle. This cell line expresses an enhanced green fluorescent protein (EGFP) fused with the Kleisin-beta protein, a crucial component of the cohesin complex that is vital for sister chromatid cohesion. The expression of EGFP-tagged Kleisin-beta allows for real-time visualization of cohesin dynamics and localization throughout the cell cycle, facilitating detailed analyses of chromosome structure and function in a cellular context.

This cell model is typically utilized in research focusing on the mechanisms of mitotic and meiotic chromosome segregation, particularly looking at how cohesin's regulation influences genetic stability and cell division. The fluorescent tagging of Kleisin-beta enables the investigation of its interaction with other cohesin components and chromosomal proteins, providing insights into the spatial and temporal assembly of cohesin on chromosomes. The use of this cell line extends to studies of genetic disorders and cancers where cohesin function is disrupted, offering a valuable tool for understanding pathogenesis and developing therapeutic strategies.

Organism

Human

Tissue

Cervix

Disease

Carcinoma

Synonyms

HeLa Kyoto EGFP Kleisin-b, HeLa Kyoto Kleisin-beta EGFP

Age

30 years

Gender

Female

Ethnicity

African American

Morphology

Epithelial-like cells with mosaic stone shape

Growth properties

Monolayer, adherent

Citation

HK EGFP-Kleisin-beta (Cytion catalog number 300674)

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Biosafety level 1

NCBI_TaxID 9606

CellosaurusAccession CVCL_1D64

Depositor The Ellenberg Lab (EMBL)

GMO Status GMO-S1: This HeLa Kyoto line contains an EGFP-kleisin-beta construct for live-cell studies of cohesin and chromosome architecture. This classification applies only within Germany and may differ elsewhere.

Protein expression EGFP-Kleisin- β : Location/Gene: 1..589 / Pcmv, 619..645 / Flag-tag, 661..1368 / GFP, 1393..3206 / Kleisin Beta, 4474..5268 KanR/NeoR

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 1×10^4 cells/cm²

Fluid renewal 2 to 3 times per week

Post-Thaw 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.

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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 $300 \times g$ 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.

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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.