Product sheet

HK EGFP-Kleisin-beta Cells | 300674

General information

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

Characteristics

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

Regulatory Data

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.

Biomolecular Data

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

Handling

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.
Flask Coating	None
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.

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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.

Quality Control & Molecular Analysis

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.