

HK EGFP-LaminB1/H2B-mCherry Cells | 300919**Renseignements généraux****Description**

The HK EGFP-LaminB1/H2B-mCherry cell line is an HeLa Kyoto derived in vitro model designed for real-time visualization of chromatin dynamics and nuclear architecture in living cells. This cell line expresses two fluorescent protein fusions: EGFP (enhanced green fluorescent protein) fused with Lamin B1, and mCherry (a red fluorescent protein) fused with histone H2B. The fusion of EGFP with Lamin B1 allows for the observation of the nuclear envelope and nuclear lamina, structures critical for maintaining the integrity and functionality of the nucleus. Lamin proteins are type V intermediate filament proteins that form a meshwork underlying the inner nuclear membrane, playing key roles in nuclear stability, chromatin organization, and gene regulation.

On the other hand, the mCherry-tagged histone H2B enables the visualization of chromatin within the nucleus. Histones are fundamental components of the nucleosome, involved in the organization of DNA into chromatin, making them crucial for DNA replication, repair, and transcription. The mCherry tag on H2B provides a vivid red fluorescence that contrasts with the green fluorescence of EGFP, allowing for simultaneous dual-imaging of the nuclear structure and chromatin in live-cell experiments. This cell line is commonly used in studies focusing on nuclear mechanics, mitosis, and genome stability, providing a dynamic view of cellular processes that are otherwise difficult to observe in real time.

Organism Human**Tissue** Cervix**Disease** Carcinoma**Synonyms** HeLa Kyoto EGFP-LaminB1 and H2B-mCherry**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 EGFP-LaminB1/H2B-mCherry (Cytion catalog number 300919)

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Biosafety level 1**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_UR41**Depositor** The Ellenberg Lab (EMBL)**GMO Status** GMO-S1: This HeLa Kyoto line contains EGFP-Lamin B1 and H2B-mCherry constructs for imaging nuclear envelope and chromatin organization. This classification applies only within Germany and may differ elsewhere.**Données biomoléculaires****Protein expression** EGFP-LaminB1/H2B-mCherry**Products** Histone H2B**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** 1×10^4 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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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.