

HK EGFP-LaminA/H2B-mCherry Cells | 300921

Renseignements généraux

Description

The HK EGFP-LaminA/H2B-mCherry cell line is a genetically engineered HeLa Kyoto derived cell model developed to facilitate advanced studies in nuclear dynamics and chromatin organization within living cells. This cell line expresses two fusion proteins: EGFP (enhanced green fluorescent protein) fused with Lamin A, and mCherry (a red fluorescent protein) fused with Histone H2B. The EGFP-Lamin A fusion highlights the nuclear envelope and allows for the visualization of nuclear architecture changes during cell cycle progression or under various experimental conditions. Meanwhile, the H2B-mCherry fusion protein binds to DNA and provides a vivid red fluorescence that marks chromatin, enabling real-time observation of chromosomal processes during mitosis and interphase.

These cells are invaluable for real-time imaging applications, including studies on nuclear integrity, DNA replication, and cellular aging, as well as research into diseases where nuclear architecture is disrupted, such as cancer and laminopathies. The dual-color fluorescence feature of this cell line allows for simultaneous visualization of both the nuclear envelope and chromatin, facilitating a comprehensive understanding of nuclear-cytoplasmic interactions and the spatiotemporal organization of chromatin. Such capabilities make it a critical tool for molecular biology research and cellular biophysics, providing insights into the mechanics of gene expression regulation, nuclear organization, and the cell cycle.

Organism Human

Tissue Cervix

Disease Carcinoma

Synonyms HeLa Kyoto EGFP-LaminA 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

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Citation HK EGFP-LaminaA/H2B-mCherry (Cytion catalog number 300921)

Biosafety level 1

NCBI_TaxID 9606

CellosaurusAccession CVCL_1D62

Depositor The Ellenberg Lab (EMBL)

GMO Status GMO-S1: This HeLa Kyoto line contains EGFP-Lamin A and H2B-mCherry constructs enabling dual-color imaging of nuclear lamina and chromatin. This classification applies only within Germany and may differ elsewhere.

Données biomoléculaires

Protein expression EGFP-LaminaA/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

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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.

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% CO₂, 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.

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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.

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.