# HK EGFP-alpha-tubulin/H2B-mCherry

300670

## GENERAL INFORMATION

### Description

The HK EGFP-alpha-tubulin/H2B-mCherry HeLa Kyoto cell line is a meticulously engineered model designed for detailed visualization of cellular processes. This clonal line has been stably transfected to express two fluorescent protein fusions that enable real-time imaging of both chromatin and the microtubular network. The red fluorescent protein mCherry is fused to the core histone protein H2B, creating H2B-mCherry. This fusion protein is expressed from the pH2B-mCherry-IRES-neo3 plasmid and serves as a chromatin marker, highlighting the nuclear DNA in live-cell imaging and facilitating studies on chromatin dynamics and nuclear architecture.
Additionally, this cell line expresses monomeric enhanced GFP (Green Fluorescent Protein) fused to α-tubulin, introduced via the pmEGFP-α-tubulin-IRES-puro2b plasmid. The GFP-α-tubulin fusion provides a vivid green fluorescence that outlines the microtubule structures within the cell. This feature is crucial for studying microtubule organization, dynamics, and their role in cell division and intracellular transport. The stable integration of these constructs allows for continuous, long-term observation of these cellular components without the need for repeated transfection, thus reducing variability and enhancing the reliability of experimental results. Drug resistance selection following transfection ensures the stability and uniformity of expression among the cells in this line.

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| **Tissue** | Cervix |
| **Disease** | Carcinoma |

### Synonyms

- HeLa Kyoto EGFP-a-tubulin/H2B-mCherry
- HeLa H2B-mRFP and mEGFP-alpha-tubulin

## CLASSIFICATION

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| **Organism** | Human |
| **Sex** | Female |
| **Growth properties** | Adherent |
| **GMO status** | GMO-S1 |

### GMO status details

GMO-S1: This HeLa Kyoto line contains EGFP-α-tubulin and H2B-mCherry constructs for simultaneous imaging of microtubules and chromatin. This classification applies only within Germany and may differ elsewhere.

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| **Biosafety level** | 1 |

## CHARACTERISTICS

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| **Morphology** | Epithelial-like cells with mosaic stone shape |
| **Doubling time** | 24 hours |

## IDENTIFIERS

### Citation

HK EGFP-alpha-tubulin/H2B-mCherry (Cytion catalog number 300670)

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| **NCBI TaxID** | 9606 |

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| **Cellosaurus** | CVCL_L802 |
| **Depositor** | The Ellenberg Lab (EMBL) |

## EXPRESSION AND SUSCEPTIBILITY

### Protein expression

EGFP-alpha-tubulin, H2B-mCherry: Location/Gene: 1..589 / Pcmv, 652..1029 H2B, 1042..1752 / mCherry, 2983..3777 / KanR/NeoR

### Viruses

Negative for HIV, HBV and HCV.

### Products

CMV Promotor, Histone H2B, Neomycin, Phosphotransferase

## HANDLING AND CULTURE

### 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)

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| **Incubation atmosphere** | 37°C, 5% $CO_2$, humidified atmosphere. |

### Supplements

Supplement the medium with 10% FBS

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| **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.

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| **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. |

### Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

## QUALITY CONTROL AND CRYOPRESERVATION

### 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.

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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. |

### 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.

### 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.

## DONOR

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| **Donor age** | 30 years |
| **Ethnicity** | African American |

## LEGAL AND SAFETY

### Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

### Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

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| **Third-party agreements apply** | Yes |