**Product sheet**

# HK EGFP-alpha-tubulin/H2B-mCherry Cells | 300670

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| **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.<br><br>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. |
| **Organism** | Human |
| **Tissue** | Cervix |
| **Disease** | Carcinoma |
| **Synonyms** | HeLa Kyoto EGFP-a-tubulin/H2B-mCherry, HeLa H2B-mRFP and mEGFP-alpha-tubulin |

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

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

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| **Biosafety level** | 1 |
| **NCBI_TaxID** | 9606 |
| **CellosaurusAccession** | CVCL_L802 |
| **Depositor** | The Ellenberg Lab (EMBL) |
| **GMO Status** | 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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| **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 |

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| **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 |
| **Doubling time** | 24 hours |
| **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/$cm^2$ |

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| **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. |
| **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.

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| **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. |

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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. |

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| **Sterility** | Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.<br><br>To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections. |