Product sheet

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

General information

Description	This HeLa reporter cell line stably expresses a red chromatin marker (core histone 2B fused to monomeric Cherry; H2B-mCherry; transfection plasmid: pH2B-mCherry-IRES-neo3) and a marker for microtubules (monomeric enhanced GFP-?-tubulin; transfection plasmid: pmEGFP-a-tubulin-IRES-puro2b). This clonal stable cell line was generated by transfection of a circular plasmid followed by drug resistance selection.
Organism	Human
Tissue	Cervix
Disease	Carcinoma
Synonyms	HeLa Kyoto EGFP-a-tubulin/H2B-mCherry, HeLa H2B-mRFP and mEGFP-alpha-tubulin

Characteristics

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

Identifiers / Biosafety / Citation

Citation	HK EGFP-alpha-tubulin/H2B-mCherry (Cytion catalog number 300670)
Biosafety level	1
Depositor	Dr. J. Ellenberg, EMBL Heidelberg

Expression / Mutation

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

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Viruses	Negative for HIV, HBV and HCV.
Products	CMV Promotor, Histone H2B, Neomycin, Phosphotransferase

Handling

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
Medium supplements	Supplement the medium with 10% FBS
Passaging solution	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.
Split ratio	A ratio of 1:3 is recommended
Seeding density	1 x 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week
Freezing 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	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

Handling of cryopreserved cultures

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. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
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.

Quality control / Genetic profile / HLA

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.