

## HK EGFP-LaminB1/H2B-mCherry growing culture | 330919

### General information

<b>Description</b>	This clonal stable cell line was generated by transfection of a circular plasmid followed by drug resistance selection.
<b>Organism</b>	Human
<b>Tissue</b>	Cervix
<b>Disease</b>	Carcinoma
<b>Synonyms</b>	HeLa Kyoto EGFP-LaminB1 and H2B-mCherry

### Characteristics

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

### Identifiers / Biosafety / Citation

<b>Citation</b>	HK EGFP-LaminB1/H2B-mCherry (Cytion catalog number 300919)
<b>Biosafety level</b>	1
<b>Depositor</b>	Dr. J. Ellenberg, EMBL Heidelberg

### Expression / Mutation

<b>Protein expression</b>	EGFP-LaminB1/H2B-mCherry
<b>Products</b>	Histone H2B

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### Handling

<b>Culture Medium</b>	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO <sub>3</sub> , w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
<b>Medium supplements</b>	Supplement the medium with 10% FBS
<b>Passaging solution</b>	Accutase
<b>Subculturing</b>	Remove medium and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add Accutase (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
<b>Split ratio</b>	A ratio of 1:3 is recommended
<b>Seeding density</b>	1 x 10 <sup>4</sup> cells/cm <sup>2</sup>
<b>Fluid renewal</b>	2 to 3 times per week
<b>Freezing recovery</b>	After thawing, plate the cells at 5 x 10 <sup>4</sup> cells/cm <sup>2</sup> and allow the cells to recover from the freezing process and to adhere for at least 24 hours.
<b>Freeze medium</b>	CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)
<b>Handling of cryopreserved cultures</b>	HK EGFP-LaminB1/H2B-mCherry cells are shipped in a deep-frozen state on dry ice. Upon receipt, confirm that the vial remains frozen. For storage, place the cryovial immediately at temperatures below -150 degrees. If you plan to culture the cells immediately, swiftly thaw the vial by shaking it in a 37 degrees water bath with clean water and an antimicrobial agent for 40-60 seconds. Remove the vial once a small ice clump persists, ensuring it remains cold. Proceed with all subsequent steps under aseptic conditions. In a sterile flow hood, disinfect the cryovial with 70% ethanol. Then, gently open the vial and transfer the cell suspension into a 15 ml centrifuge tube pre-filled with 8 ml of room temperature culture medium. Gently mix the cells. For cell separation, centrifuge at 300 x g for 3 minutes and dispose of the supernatant. Skipping centrifugation is optional, although any residual freezing medium should be removed after 24 hours. Resuspend the pellet gently in 10 ml of fresh culture medium and divide between two T25 culture flasks. Follow the subculture protocol for subsequent steps.

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#### **Handling of proliferating cultures**

One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in a 50 ml centrifuge tube. Spin down the collected medium at 300 x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to a T25 cell culture flask. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Examine cell morphology and confluency using a microscope. Finally, incubate the flasks at 37 degrees Celsius for at least 24 hours.

#### **Quality control / Genetic profile / HLA**

#### **Sterility**

Mycoplasma contamination is rigorously 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.