

## HK-CRISPR-mEGFP-Nup358 no.97 growing culture | 331575

### General information

<b>Organism</b>	Human
<b>Tissue</b>	Endocervix
<b>Disease</b>	Adenocarcinoma

### 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>	Adherent

### Identifiers / Biosafety / Citation

<b>Citation</b>	HK-CRISPR-mEGFP-Nup358 no.97 (Cytion catalog number 301575)
<b>Biosafety level</b>	1
<b>Depositor</b>	Dr. J. Ellenberg, EMBL Heidelberg

### Expression / Mutation

<b>Products</b>	EGFP (Enhanced Green Fluorescent Protein)
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### Handling

<b>Culture Medium</b>	DMEM
<b>Medium supplements</b>	10% FBS, 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

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**Passaging solution** Accutase

**Subculturing** 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 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.

**Split ratio** A ratio of 1:3 is recommended

**Fluid renewal** 2 to 3 times per week

**Freeze medium** CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100)

**Handling of cryopreserved cultures** The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial must be stored below -150 degree Celsius after arrival. If immediate culturing is intended, please follow the below instructions: Quickly thaw by rapid agitation in a 37 degree Celsius water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. A small ice clump should still remain and the vial should still be cold. From now on, all operations should be carried out under aseptic conditions. Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300 x g for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later. Resuspend the cells carefully in 10 ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the subculture section.

**Handling of proliferating cultures** One or two cell culture flasks come filled with cell culture medium. Collect the entire medium in 1 or 2 x 50 ml centrifuge tubes, respectively. Carefully add 5 ml of cell culture medium to each T25 cell culture flask. Control the cell morphology and confluency under the microscope. Incubate at 37 degree Celsius for a minimum of 24 hours. 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. Incubate at 37 degree Celsius for a minimum of 24 hours.

## Quality control / Genetic profile / HLA

**Sterility** Mycoplasma contaminations are ruled out through PCR-based and luminescence-based mycoplasma assays. The absence of bacterial, fungal or yeast contamination is controlled through daily visual cell monitoring.