

**NRK-EGFP-H2B Cells | 500724**

**General information**

<b>Description</b>	This clonal stable cell line was generated by transfection of a circular plasmid followed by drug resistance selection. Add G418 to culture medium at a final concentration of 0.5 mg/ml.
<b>Organism</b>	Rat
<b>Tissue</b>	Kidney
<b>Synonyms</b>	NRK EGFP-H2B

**Characteristics**

<b>Morphology</b>	Fibroblast-like cells with fusiform shape
<b>Growth properties</b>	Monolayer, adherent

**Identifiers / Biosafety / Citation**

<b>Citation</b>	NRK-EGFP-H2B (Cytion catalog number 500724)
<b>Biosafety level</b>	1
<b>Depositor</b>	Dr. J. Ellenberg, EMBL Heidelberg

**Expression / Mutation**

<b>Receptors expressed</b>	Epidermal growth factor (EGF), multiplication stimulating activity (MSA)
<b>Protein expression</b>	EGFP-H2B: Location/Gene: 1..589 / Pcmv, 613..1329 / EGFP, 1387..1764 / H2B, 3001..3795 / KanR/NeoR
<b>Products</b>	Epidermal growth factor (EGF), multiplication stimulating activity (MSA), CMV Promotor Histone H2B, Neomycin, Phosphotransferase

**Handling**

<b>Culture Medium</b>	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)
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**Medium supplements** Supplement the medium with 10% FBS, 0.5 mg/ml G418

**Passaging solution** Accutase

**Subculturing** Discard the old medium and wash the cells with PBS. Add a freshly prepared 0.025% trypsin/0.02% EDTA solution heated to 37 degrees Celsius and wait until the cells detach, which usually takes about 5 minutes. Neutralize the trypsin by adding fresh medium, then transfer the cell mixture to a tube and centrifuge. After centrifugation, remove the supernatant, resuspend the cell pellet in fresh culture medium, and transfer the suspension to new flasks. Incorporate G418 into the culture medium to achieve a final concentration of 0.5 mg/ml

**Split ratio** A ratio of 1:3 to 1:4 is recommended

**Seeding density** 2 to 4 x 10<sup>4</sup> cells/cm<sup>2</sup>

**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** NRK-EGFP-H2B 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.

## 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.