

**NRK-EGFP2-Nup50 Cells | 500726****General information****Description**

The NRK-EGFP2-Nup50 cell line is a clonal stable cell line derived from normal rat kidney (NRK) cells. This cell line was generated through the transfection of a circular plasmid containing the gene encoding the fusion protein of Enhanced Green Fluorescent Protein (EGFP) and Nucleoporin 50 (Nup50), followed by drug resistance selection. As a result, approximately 50% of the cells express the EGFP3-Nup50 fusion protein, which enables the visualization and tracking of Nup50 within the cellular environment.

Nup50 is a critical component of the nuclear pore complex, which is responsible for regulating the transport of molecules between the nucleus and cytoplasm. The EGFP3 tag allows for live-cell imaging and other fluorescence-based techniques to study the localization, dynamics, and interactions of Nup50. Despite being a stable cell line, the NRK-EGFP2-Nup50 cells exhibit some variegation, indicating variability in the expression levels of the EGFP3-Nup50 fusion protein among the cells.

This cell line is particularly valuable for research focused on nucleocytoplasmic transport, nuclear pore complex dynamics, and the functional role of Nup50 in various cellular processes. The NRK-EGFP2-Nup50 cells are suitable for a range of experimental approaches, including fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS), and other advanced microscopy techniques. These studies can provide insights into the molecular mechanisms of nuclear transport and contribute to the understanding of diseases associated with nuclear transport dysfunction, such as certain cancers and neurodegenerative disorders.

**Organism** Rat**Tissue** Kidney**Synonyms** NRK EGFP2-Nup50**Characteristics****Breed/Subspecies** OsborneMendel**Morphology** Fibroblast-like cells with fusiform shape**Growth properties** Monolayer, adherent**Regulatory Data****Citation** NRK-EGFP2-Nup50 (Cytion catalog number 500726)**Biosafety level** 1

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**NCBI\_TaxID** 10116**CellosaurusAccession** CVCL\_AV93**Depositor** The Ellenberg Lab (EMBL)**Biomolecular Data****Receptors expressed** Epidermal growth factor (EGF), multiplication stimulating activity (MSA)**Protein expression** EGFP3-Nup50**Products** NUP50 (Nucleoporin 50)**Handling****Culture Medium** DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO<sub>3</sub>, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)**Supplements** Supplement the medium with 10% FBS, 0.5 mg/mL G418**Dissociation Reagent** 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**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** 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.

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### 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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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  $300 \times g$  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.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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**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.