Product sheet

NRK-EGFP3-Seh1 Cells | 500731

General information

Description	The NRK-EGFP3-Seh1 cell line is a clonal stable line derived from normal rat kidney (NRK) cells. This cell line was generated through the transfection of a circular plasmid encoding the EGFP3-Seh1 fusion protein. Following transfection, cells were selected for drug resistance, ensuring the establishment of a stable population expressing the desired construct. Approximately 50% of the cells in this population express EGFP3-Seh1, a fusion protein combining enhanced green fluorescent protein (EGFP) with Seh1, a protein component of the nuclear pore complex. The presence of EGFP facilitates visualization and tracking of the fusion protein within the cells, enabling researchers to study the dynamics and function of Seh1 in various cellular processes. However, the expression of EGFP3-Seh1 in this cell line exhibits some variegation, indicating variability in expression levels among individual cells within the population. This cell line is particularly useful for studies involving nuclear pore complex assembly, nucleocytoplasmic transport, and the role of Seh1 in these processes. The fluorescence provided by EGFP allows for live-cell imaging and real-time analysis of protein localization and interactions, making NRK-EGFP3-Seh1 a valuable tool for cell biology and molecular research.
Organism	Rat
Tissue	Kidney
Synonyms	NRK EGFP3-Seh1

Characteristics

Breed/Subspecies	OsborneMendel
Morphology	Fibroblast-like cells with fusiform shape
Growth properties	Monolayer, adherent

Regulatory Data

Citation	NRK-EGFP3-Seh1 (Cytion catalog number 500731)
Biosafety level	1
NCBI_TaxID	10116

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CellosaurusAccession	CVCL_AV94
Depositor	The Ellenberg Lab (EMBL)

Biomolecular Data

Receptors expressed	Epidermal growth factor (EGF), multiplication stimulating activity (MSA)
Protein expression	EGFP3-Seh1
Products	Seh1 (SEH1 Like Nucleoporin)

Handling

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, 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	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.
Seeding density	2 to 4 x 10^4 cells/cm^2
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.

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°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. 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°C, 5% 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 °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 °C. Storage at −80 °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.