

NRK-EGFP3-Seh1 growing culture | 550731

General information

Description	This clonal stable cell line was generated by transfection of a circular plasmid (see below) followed by drug resistance selection. About 50% of cells express EGFP3-Seh1. This clonal stable cell line shows some variegation.
Organism	Rat
Tissue	Kidney
Synonyms	NRK EGFP3-Seh1

Characteristics

Morphology	Fibroblast-like cells with fusiform shape
Growth properties	Monolayer, adherent

Identifiers / Biosafety / Citation

Citation	NRK-EGFP3-Seh1 (Cytion catalog number 500731)
Biosafety level	1
Depositor	Dr. J. Ellenberg, EMBL Heidelberg

Expression / Mutation

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: 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 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 10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300 g, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.

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

Seeding density 2 to 4 x 10⁴ cells/cm²

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-EGFP3-Seh1 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.

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.