

**Designation: NRK-EGFP3-Seh1**

CLS order number: Cryovial: 500731
Vital: 550731

Origin and General Characteristics

Depositor:	Dr Jan Ellenberg, EMBL Heidelberg.
Fluorescent marker:	EGFP3-Seh1
Percentage of expressing cells:	About 50%.
Expression level:	This clonal stable cell line shows some variegation.
Morphology:	Fibroblast-like cells with fusiform shape.
Cell type:	NRK, normal rat kidney cells.
Growth Properties:	Monolayer, adherent
Description:	This clonal stable cell line was generated by transfection of a circular plasmid (see below) followed by drug resistance selection.

Culture Conditions and Handling

Culture Medium:	DMEM medium supplemented with 4.5g/L glucose, 2 mM L-glutamine and 10% fetal bovine serum (MG-30, CLS order number 820300). The addition of 100U/mL streptomycin and 100µg/mL penicillin is optional.
Culture conditions:	37°C, 5% CO ₂ , 95% humidity.
Drug resistance:	Add G418 to culture medium at a final concentration of 0.5mg/ml.
Subculturing:	Remove medium and rinse with PBS. Add fresh 0.025% trypsin/0.02% EDTA solution at 37°C until cells detach (typically ~5 min). To remove trypsin, add fresh medium, transfer to a tube and centrifuge. Aspirate the supernatant, resuspend the cell pellet in culture medium and dispense into new flasks.
Split Ratio:	A ratio of 1:3 to 1:4 is recommended; minimum seeding density 2-4x10 ⁴ cells/cm ² .
Medium Renewal:	2 to 3 times weekly
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria specific PCR: negative
Biosafety Level:	1

Special Features of the Cell Line

Receptors Expressed: Epidermal growth factor (EGF); multiplication stimulating activity (MSA)

References:

Rabut, G., Doye, V. & Ellenberg, J. Mapping the dynamic organization of the nuclear pore complex inside single living cells. Nat Cell Biol. 2004 Nov; 6(11): 1114-21. Epub 2004 Oct 24.

Permit: EMBLEM MTA is required for the transfer of this CLS material.

Warning: The identity of the parental cell line has not been tested. The sequence of the plasmid used to generate this cell line is available but the copy number integrated in the genome has not been tested, the insertion site has not been determined and the integrated plasmids have not been sequence verified. We make no warranties of any kind about the identity of the parental cell line, the copy number and completeness or accuracy of sequences of integrated plasmids. Any reliance you place on this cell line is therefore strictly at your own risk.

Recommendations for handling of adherent cell cultures following delivery

Cryopreserved cells

If immediate culturing is not intended, the cryovial(s) must be stored in liquid nitrogen (-196°C) or at least at -80°C after arrival.

If immediate culturing is intended, please follow these instructions:

Quickly thaw by rapid agitation in a 37°C 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. Note: 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 300xg 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 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section.

Proliferating Cultures

The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit.

Remove the entire medium except for a sufficient volume to cover the floor of the flask. Incubate at 37°C for 24 hrs.

Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred remove the entire content of the flask and centrifuge at 300x g for 5 minutes. Take off the supernatant, resuspend the cells in 10 ml of culture medium and transfer the entire cell suspension into cell culture flasks of suitable size (do not seed in more than 1 T75 flask).

Safety precautions for frozen cell lines

If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed:

- Protective gloves and clothing should be used and a facemask or safety goggles must be worn when storing and/or thawing the cryovial.
- The removal of a cryovial from liquid nitrogen can result in the explosion of the cryovial creating flying fragments.

References: Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.