Designation: **NRK-EGFP2-Nup50**

CLS order number: Cryovial: 500726
Vital: 550726

Origin and General Characteristics	
Depositor:	Dr Jan Ellenberg, EMBL Heidelberg.
Fluorescent marker:	EGFP2-Nup50
Percentage of expressing cells:	Close to 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.
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.
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
Safety precautions:	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 transferring frozen samples into or removing from the liquid nitrogen tank. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments. 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.
Special Features of the Cell Line	
Receptors Expressed:	Epidermal growth factor (EGF); multiplication stimulating activity (MSA)
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.
Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

Recommendations for handling of adherent cell cultures following delivery	
Cryopreserved cells	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial(s) must be stored below -150°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, 2xT25, come filled with cell culture medium. Collect the entire medium in 2x 50 ml centrifuge tubes. Carefully add 5 ml of cell culture medium to each of the two T25 cell culture flasks. Control the cell morphology and confluency under the microscope. Incubate at 37°C for a minimum of 24 hrs. Spin down the collected medium at 300x 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 1xT25 cell culture. Incubate at 37°C for a minimum of 24 hrs.

Warranty:	CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed.
Disclaimer:	The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term ‘Commercial use’ shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale.