Designation: **U-2 OS-CRISPR-NUP96-SNAP clone #33**

CLS order number: Cryovial: 300444
Vital: 330444

Origin and General Characteristics	
Depositor:	Dr Jan Ellenberg, EMBL Heidelberg.
Organism:	*Homo sapiens* (Human)
Fluorescence Marker:	NUP96-SNAP (nuclear pore complex protein 96, SNAP-tag)
Percentage of expressing cells:	100% (according to depositor)
Expression level:	Endogenous expression level.
Production:	This clonal stable cell line was generated by CRISPR-Cas9D10A nickase-assisted genome editing.
Morphology:	Epithelial
Cell type:	Osteosarcoma
Growth Properties:	Monolayer, adherent
References:	Jervis Vermal Thevathasan, Maurice Kahnwald, Konstanty Cieśliński, Philipp Hoess, Sudheer Kumar Peneti, Manuel Reitberger, Daniel Heid, Krishna Chaitanya Kasuba, Sarah Janice Hoerner, Yiming Li, Yu-Le Wu, Markus Mund, Ulf Matti, Pedro Matos Pereira, Ricardo Henriques, Bianca Nijmeijer, Moritz Kueblbeck, Vilma Jimenez Sabinina, Jan Ellenberg and Jonas Ries. Nuclear pores as versatile reference standards for quantitative superresolution microscopy. Nat.Methods 2019 Oct; 16(10):1045-1053.
Citation:	When using this cell line in publications please cite as: U-2 OS-CRISPR-NUP96-SNAP clone #33 (300444, CLS GmbH) together with the reference above.
Warning:	We make no warranties of any kind about the identity of the parental cell line. Any reliance you place on this cell line is therefore strictly at your own risk.
Culture Conditions and Handling	
Culture Medium:	McCoy's 5a supplemented with 2 mM L-glutamine, 100 U/mL streptomycin, 100 µg/mL penicillin, 1 mM sodium pyruvate, 1% MEM non essential amino acids and 10% fetal bovine serum.
Culture conditions:	37°C, 5% CO_2, 95% humidity.
Drug resistance:	No drug resistance
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 8-10 minutes. Carefully resuspend the cells with medium (5-10 ml), centrifuge for 3 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split ratio:	A ratio of 1:4 to 1:6 is recommended
Seeding density:	1 to $2x10^4$ cells/cm^2
Fluid Renewal:	Every 2 to 3 days

Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml CLS Cell Lines Service GmbH).
Sterility:	Mycoplasma-specific PCR: negative; Cell based assay (Plasmotest): 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.
Permit:	**This product is subject to the EMBLEM MTA, which must be completed and signed ahead of shipment. Please send your request to: info@clsgmbh.de**
Special Features of the Cell Line	
Fluorescence:	The cell line is not fluorescent by itself. For imaging a fluorescent dye to label the SNAP tag must be added. Example protocol (according to depositor): 1) Add SNAP TMR-Star (S9105, NEB) at 1µM final concentration to cells in growing medium; incubate for 15 minutes at 37°C, 5% CO_2. 2) Wash 5x with growing medium. 3) Leave the cells in growing medium for 15 minutes at 37°C, 5% CO_2. 4) Wash 5x with growing medium. 5) Perform microscopy (Excitation: 554nm; Emission: 580nm) The amount of background depends on the wash procedure which can be done longer to have cleaner signal.

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. This product from CLS has been produced under license for third parties. The customer shall take the submitted licensing terms and conditions of third parties into consideration and may only make use of such within the scope of the rights granted therein.