Product sheet

U2OS-CRISPR-NUP96-mMaple Cells | 300461

General information

Description	The U-2 OS-CRISPR-NUP96-mMaple is a genetically engineered osteosarcoma cell line derived from the human U-2 OS cell line, known for its robust growth characteristics and utility in various biological studies. This particular clone has been modified using CRISPR/Cas9 gene editing technology to incorporate mMaple, a photoconvertible fluorescent protein, into the NUP96 gene. The mMaple protein allows for advanced imaging techniques such as live-cell imaging and super-resolution microscopy, providing dynamic insights into the nuclear pore complex (NPC) behavior and cellular import-export mechanisms through the nuclear envelope. The NUP96 gene, which encodes a crucial component of the NPC, is vital for nucleocytoplasmic transport. Alteration of NUP96 can affect not only transport mechanisms but also overall nuclear architecture and function. This cell line thus serves as an excellent model for studying NPC-related pathologies and the role of nuclear transport in cellular metabolism and signaling. The integration of mMaple into NUP96 permits real-time tracking and visualization of NUP96 dynamics in vivo, making it an indispensable tool for researchers focused on cell nucleus studies and those exploring the implications of NPC dysfunctions in diseases such as cancer and viral infections. As a specialized tool, U-2 OS-CRISPR-NUP96-mMaple clone no.16 supports high-resolution imaging and provides substantial data regarding the spatial and temporal distribution of NPC components. It is particularly valuable for experiments requiring detailed analysis of gene expression, protein localization, and nuclear transport under physiological and pathological conditions, facilitating a deeper understanding of cellular processes at the molecular level.
Organism	Human
Tissue	Bone
Disease	Osteosarcoma

Characteristics

Age	15 years
Gender	Female
Ethnicity	Caucasian
Growth properties	Adherent

Regulatory Data

Citation	U-2 OS-CRISPR-NUP96-mMaple (Cytion catalog number 300461)

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Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_B7FK
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This human osteosarcoma cell line (U2OS-CRISPR-NUP96-mMaple, clone 16) contains a CRISPR-mediated NUP96-mMaple fusion enabling photoconvertible labeling of nuclear pore structures. The construct is stably present. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Protein expression	NUP96-mMaple (endogenous nuclear pore complex protein 96, mMaple tagged)

Handling

Culture Medium	McCoys 5a, w: 3.0 g/L Glucose, w: stable Glutamine, w: 2.0 mM Sodium pyruvate, w: 2.2 g/L NaHCO3 (Cytion article number 820200a)
Supplements	Supplement the medium with 10% FBS, 1% NEAA
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	1×10^4 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.

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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.
Flask Coating	None
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.