Product sheet

U2OS-CRISPR-NUP96-mEGFP Cells | 300174

Renseignements généraux

Description	The U-2 OS-CRISPR-NUP96-mEGFP is a genetically modified cell line derived from the human osteosarcoma U-2 OS parent line. This cell line incorporates a targeted insertion of the monomeric Enhanced Green Fluorescent Protein (mEGFP) tag at the NUP96 gene locus, achieved through the CRISPR-Cas9 gene-editing technology. NUP96, part of the nuclear pore complex, is essential for nuclear transport, and its fusion with mEGFP allows for real-time visualization of nuclear pore dynamics under fluorescent microscopy, providing valuable insights into nuclear transport mechanisms and nucleocytoplasmic trafficking. This specific clone, numbered 195, has been selected for its stable expression of the NUP96-mEGFP fusion protein and maintains the typical characteristics of the U-2 OS lineage, including a robust cytoskeletal structure which is critical in studies related to cancer cell migration and metastasis. The application of CRISPR technology ensures precise gene editing, minimizing off-target effects which could compromise the integrity of the experimental outcomes. This makes U-2 OS-CRISPR-NUP96-mEGFP clone no.195 particularly useful for high-resolution imaging techniques and detailed cellular architecture studies, aiding in advanced research in cellular biology, cancer research, and nuclear transport phenomena.
Organism	Human
Tissue	Bone
Disease	Osteosarcoma

Caractéristiques

Age	15 years
Gender	Female
Ethnicity	Caucasian
Morphology	Epithelial-like
Growth properties	Adherent

Données réglementaires

Citation	U-2 OS-CRISPR-NUP96-mEGFP clone no.195 (Cytion catalog number 300174)
Biosafety level	1

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NCBI_TaxID	9606
CellosaurusAccession	CVCL_B7FJ
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This human osteosarcoma cell line (U2OS-CRISPR-NUP96-mEGFP, clone 195) contains a CRISPR-engineered NUP96-mEGFP fusion introduced via lentiviral delivery, enabling fluorescent tracking of nuclear pore complexes. The modification is stably integrated. This classification applies only within Germany and may differ elsewhere.

Données biomoléculaires

Protein expression	MEGFP (nuclear pore complex protein 96, mEGFP tagged)

Manipulation

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	2 to 3 x 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.

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.

Contrôle de la qualité et analyse moléculaire

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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.