Product sheet

U2OS-CRISPR-NUP96-SNAP Cells | 300444

Renseignements généraux

Description	The U-2 OS-CRISPR-NUP96-SNAP is a genetically modified osteosarcoma cell line derived from the parent U-2 OS human cell line. This cell line has been engineered through CRISPR/Cas9-mediated genome editing to incorporate a SNAP-tag at the NUP96 gene, enabling the visualization and study of nuclear pore complex dynamics. Nuclear pore complexes (NPCs) are crucial for the regulation of nucleocytoplasmic transport, and NUP96 is a significant component of the NPC, playing a pivotal role in its structural integrity and function. In U-2 OS-CRISPR-NUP96-SNAP clone no.33, the integration of the SNAP-tag at the NUP96 locus allows for specific and covalent attachment of fluorescent substrates or other chemical probes that can be used for live-cell imaging and other biochemical assays. This feature makes it an invaluable tool for investigating the molecular dynamics of nucleocytoplasmic transport, understanding NPC-related pathologies, and screening for therapeutic compounds that affect NPC function. The cell line also retains the characteristics of the parental U-2 OS line, which includes a high level of genetic stability and ease of culture, making it suitable for high-throughput screening and extended studies in cell biology. Due to the specificity of the modification at the NUP96 gene, U-2 OS-CRISPR-NUP96-SNAP clone no.33 provides a unique model for the detailed study of NPC components in the context of cellular function and dysfunction. Researchers can exploit the SNAP-tag system to selectively and rapidly label NUP96, facilitating real-time visualization of NPC dynamics under physiological and pathological conditions. This specific clone can serve as a robust platform for both basic research and applied biomedical studies, contributing significantly to the fields of cellular biology, genetics, and oncology.
Organism	Human
Tissue	Bone
Disease	Osteosarcoma

Caractéristiques

Age	15 years
Gender	Female
Ethnicity	Caucasian
Growth properties	Adherent

Données réglementaires

Citation	U-2 OS-CRISPR-NUP96-SNAP (Cytion catalog number 300444)

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Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_B7FL
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This human osteosarcoma cell line (U2OS-CRISPR-NUP96-SNAP, clone 33) contains a CRISPR-engineered NUP96-SNAP fusion facilitating SNAP-tag chemical labeling of nuclear pores. The modification is stably integrated. This classification applies only within Germany and may differ elsewhere.

Données biomoléculaires

Protein expression	NUP96-SNAP (nuclear pore complex protein 96, SNAP-tag)

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, 3.0 g/L Glucose, stable Glutamine, 2.0 mM Sodium pyruvate, 2.2 g/L NaHCO3, 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

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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.

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

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.