Product sheet

U2OS-CRISPR-NUP96-Halo Cells | 300448

General information

Description	The U-2 OS-CRISPR-NUP96-Halo is a genetically engineered cell line derived from the human osteosarcoma U-2 OS cells. This cell line has been modified using CRISPR-Cas9 technology to incorporate a HaloTag at the NUP96 gene locus. NUP96, part of the nuclear pore complex, plays a critical role in nuclear transport and cellular regulation. The introduction of the HaloTag allows for the precise visualization and biochemical characterization of NUP96's dynamics and interactions within the cell. By facilitating the covalent attachment of fluorescent ligands or other probes, the HaloTag enables real-time imaging and provides a powerful tool for studying the nuclear transport mechanisms in living cells. This particular clone, number 252, has been selected for its stable expression of the HaloTagged NUP96, ensuring consistent performance in experimental setups. This feature makes it highly suitable for high-resolution imaging techniques and molecular interaction studies, thereby supporting advanced research in cellular biology, particularly in the context of nuclear function and genetic regulation.
Organism	Human
Tissue	Bone
Disease	Osteosarcoma

Characteristics

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

Regulatory Data

Citation	U-2 OS-CRISPR-NUP96-Halo (Cytion catalog number 300448)
Biosafety level	1
NCBI_TaxID	9606

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CellosaurusAccession	CVCL_B7FI
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This human osteosarcoma cell line (U2OS-CRISPR-NUP96-Halo, clone 252) contains a CRISPR-edited NUP96-Halo fusion generated via lentiviral delivery, enabling fluorescent labeling of nuclear pore complexes. The modification is stably integrated. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Protein expression	NUP96-Halo (endogenous nuclear pore complex protein 96, Halo 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
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.