Product sheet

U2OS-CRISPR-SNAPf-Nup133 Cells | 300666

Renseignements généraux

Description	U2OS-CRISPR-SNAPf-Nup133 is a genetically engineered human osteosarcoma cell line derived from the parental U2OS background in which the endogenous NUP133 locus has been modified using CRISPR/Cas9-mediated genome editing to encode a C-terminal SNAPf tag. NUP133 is a core component of the Y-complex (NUP107-160 complex), a structural subcomplex essential for nuclear pore complex (NPC) assembly and maintenance. By introducing the SNAPf coding sequence in-frame at the endogenous locus, the fusion protein is expressed under native regulatory control, preserving physiological expression levels and subcellular localization. The SNAPf tag is a fast-labeling variant of the SNAP-tag, an engineered O6-alkylguanine-DNA alkyltransferase that covalently reacts with benzylguanine-conjugated substrates. This enables highly specific and versatile fluorescent labeling of Nup133 in live or fixed cells using cell-permeable or impermeable SNAP substrates. In U2OS-CRISPR-SNAPf-Nup133 cells, the fusion protein localizes to the nuclear envelope in a punctate pattern characteristic of nuclear pore complexes. Because tagging occurs at the endogenous locus, NPC stoichiometry and architecture are minimally perturbed, making this model suitable for quantitative super-resolution microscopy, single-molecule tracking, and kinetic analyses of NPC assembly and turnover. This cell line provides a robust platform for studying nuclear transport, nucleocytoplasmic trafficking dynamics, NPC biogenesis during interphase and post-mitotic nuclear reassembly, and structural organization of the Y-complex within the pore scaffold. The U2OS background offers flat morphology and large nuclei, facilitating high-resolution imaging. U2OS-CRISPR-SNAPf-Nup133 cells are particularly well suited for pulse-chase labeling experiments, correlative light and electron microscopy, and multicolor imaging approaches in combination with additional endogenously tagged nucleoporins or transport factors.
Organism	Human
Tissue	Bone
Disease	Osteosarcoma

Caractéristiques

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

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Données réglementaires

Citation	U2OS-CRISPR-SNAPf-Nup133 (Cytion catalog number 300666)
Biosafety level	1
NCBI_TaxID	9606
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This human osteosarcoma cell line (U2OS-CRISPR-SNAPf-Nup133) contains a CRISPR-introduced SNAPf-Nup133 fusion, enabling fluorescent tagging of the Nup133 nucleoporin. The insert is stably present. This classification applies only within Germany and may differ elsewhere.

Données biomoléculaires

Protein expression	Nup133, SNAPf-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.
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.