U2OS-CRISPR-SNAPf-Nup358/RanBP2

300663

GENERAL INFORMATION

Description

U2OS-CRISPR-SNAPf-Nup358/RanBP2 is a genome-edited human osteosarcoma cell line derived from U2OS cells in which the endogenous RANBP2 (also known as NUP358) locus has been modified by CRISPR/Cas9 to encode a SNAPf tag in-frame with the native protein. Nup358/RanBP2 is a large nucleoporin localized to the cytoplasmic filaments of the nuclear pore complex (NPC) and plays critical roles in nucleocytoplasmic transport, SUMOylation, and mitotic processes. Endogenous tagging ensures that SNAPf-Nup358 is expressed under physiological promoter control, maintaining native expression levels and minimizing artifacts associated with overexpression systems. The SNAPf tag is a fast-labeling variant of the SNAP-tag that covalently binds benzylguanine-conjugated substrates, enabling selective and stable fluorescent labeling of Nup358 in live or fixed cells. In U2OS-CRISPR-SNAPf-Nup358/RanBP2 cells, the fusion protein localizes to the nuclear envelope in a punctate distribution characteristic of cytoplasmic NPC filaments. This configuration supports high-resolution fluorescence imaging, super-resolution microscopy, pulse-chase labeling, and single-molecule tracking approaches to study NPC architecture and dynamics. The flat morphology and large nuclei of U2OS cells further facilitate quantitative imaging of nuclear envelope structures.
This model enables investigation of Nup358-specific roles in CRM1/exportin-dependent nuclear export, Ran GTPase cycle regulation, and the spatial organization of cytoplasmic transport platforms. Given the involvement of Nup358 in mitotic spindle assembly and kinetochore function, the cell line is also suitable for studying cell cycle-dependent redistribution of nucleoporins and NPC disassembly/reassembly during mitosis. U2OS-CRISPR-SNAPf-Nup358/RanBP2 provides a physiologically relevant platform for dissecting structural and functional aspects of the cytoplasmic face of the nuclear pore complex in human cells.

Tissue	Bone
Disease	Osteosarcoma
Metastatic site	Primary tumor site (bone)

Applications

Nuclear pore complex cytoplasmic filament biology; Nup358/RanBP2 in CRM1-mediated nuclear export; Ran GTPase cycle; SUMO pathway; mitotic spindle assembly; single-particle tracking; super-resolution microscopy; SNAP pulse-chase labeling; NPC cytoplasmic face architecture

CLASSIFICATION

Organism	Human
Sex	Female
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

GMO-S1: This human osteosarcoma cell line (U2OS-CRISPR-SNAPf-Nup358/RanBP2) contains a CRISPR-engineered SNAPf-Nup358/RanBP2 fusion enabling precise labeling of nuclear pore cytoplasmic fibrils. The modification is stably integrated. This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

CHARACTERISTICS

Morphology	Epithelial-like
Cell type	Epithelial cells (osteosarcoma)

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Doubling time	approx. 24 to 36 hours

IDENTIFIERS

Citation

U2OS-CRISPR-SNAPf-Nup358/RanBP2 (Cytion catalog number 300663)

NCBI TaxID	9606
Cellosaurus	Not assigned (CRISPR-modified U2OS derivative; parental U2OS CVCL_0042)
Depositor	The Ellenberg Lab (EMBL)

EXPRESSION AND SUSCEPTIBILITY

Protein expression

Nup358/RanBP2, SNAPf-tag

HANDLING AND CULTURE

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)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

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.

Split ratio	1 to 3
Seeding density	1 to 3 × 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

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.

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.

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.

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.

DONOR

Donor age	15 years
Ethnicity	Caucasian

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Third-party agreements apply	Yes