U2OS-CRISPR-SNAPf-SEH1

300664

GENERAL INFORMATION

Description

U2OS-CRISPR-SNAPf-SEH1 is a genome-edited human osteosarcoma cell line derived from U2OS cells in which the endogenous SEH1L (SEH1) gene has been modified using CRISPR/Cas9 technology to encode an in-frame SNAPf tag. SEH1 is a component of the Y-complex (also known as the NUP107-160 complex), a core structural module of the nuclear pore complex (NPC) that contributes to pore scaffold assembly and stability. By inserting the SNAPf coding sequence at the endogenous locus, the tagged SEH1 protein is expressed under native regulatory control, preserving physiological expression levels and minimizing perturbations to nuclear pore composition.
The SNAPf tag is an engineered, fast-reacting variant of the SNAP-tag that covalently binds benzylguanine-conjugated substrates, allowing selective and stable fluorescent labeling in live or fixed cells. In U2OS-CRISPR-SNAPf-SEH1 cells, the fusion protein localizes to the nuclear envelope in a punctate pattern characteristic of NPC distribution. Because labeling occurs at endogenous protein levels, this system is well suited for quantitative fluorescence microscopy, super-resolution imaging, and single-particle tracking analyses aimed at dissecting NPC organization and stoichiometry. The flat morphology and large nuclei of U2OS cells further facilitate high-resolution visualization of nuclear envelope structures.
SEH1 participates in NPC biogenesis and has also been implicated in kinetochore-associated processes during mitosis. Accordingly, this cell line provides a robust platform for investigating cell cycle-dependent NPC assembly and disassembly, spatial organization of the Y-complex within the pore scaffold, and potential dual roles of SEH1 at the nuclear envelope and mitotic kinetochores. U2OS-CRISPR-SNAPf-SEH1 enables mechanistic studies of nuclear pore architecture and dynamics under physiologically relevant expression conditions.

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

Applications

Y-complex/NUP107-160 complex biology; SEH1 in NPC scaffold assembly; kinetochore-associated NPC components; NPC stoichiometry; SNAP pulse-chase labeling; super-resolution microscopy; NPC biogenesis; mitotic NPC disassembly and reassembly

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-SEH1) contains a CRISPR-mediated SNAPf-SEH1 fusion that allows selective labeling of the SEH1 nucleoporin. The modification is stably present. This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

CHARACTERISTICS

Morphology	Epithelial-like
Cell type	Epithelial cells (osteosarcoma)
Doubling time	approx. 24 to 36 hours

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IDENTIFIERS

Citation

U2OS-CRISPR-SNAPf-SEH1 (Cytion catalog number 300664)

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

EXPRESSION AND SUSCEPTIBILITY

Protein expression

SEH1, 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