Product sheet

HK-CRISPR-mEGFP-RanBP2/Nup358 Cells | 301575

General information

Description	The HK-CRISPR-mEGFP-Nup358 cell line is a genetically engineered derivative of HeLa Kyoto cells, known for their robustness and widespread use in scientific research. This cell line has been modified using CRISPR-Cas9 technology to express mEGFP (monomeric Enhanced Green Fluorescent Protein) tagged Nup358, a crucial component of the nuclear pore complex (NPC). Nup358, also known as RanBP2, plays a significant role in nucleocytoplasmic transport, mitotic spindle assembly, and other cellular processes. The mEGFP tag allows for the visualization of Nup358, facilitating real-time observation of its dynamics and interactions within the cell. HeLa Kyoto cells, a subline of the original HeLa cells, are characterized by their adaptability and stable growth in culture. The CRISPR-Cas9 system in this cell line enables precise genomic editing, ensuring the mEGFP tag is accurately fused to the Nup358 protein without disrupting its function. This makes the HK-CRISPR-mEGFP-Nup358 cell line a valuable tool for studying the structural and functional aspects of the nuclear pore complex. Researchers can use this cell line to gain insights into the mechanisms governing nucleocytoplasmic transport and the role of Nup358 in cellular homeostasis and disease states, such as cancer and viral infections.
Organism	Human
Tissue	Endocervix
Disease	Adenocarcinoma

Characteristics

Age	30 years
Gender	Female
Ethnicity	African American
Morphology	Epithelial-like cells with mosaic stone shape
Growth properties	Adherent

Regulatory Data

Citation	HK-CRISPR-mEGFP-Nup358 (Cytion catalog number 301575)
Biosafety level	1
NCBI_TaxID	9606

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CellosaurusAccession	CVCL_B7FS
Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This HeLa Kyoto line contains a CRISPR-integrated mEGFP tag at the RanBP2/Nup358 locus, enabling visualization of cytoplasmic filaments of the nuclear pore. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Products	EGFP (Enhanced Green Fluorescent Protein)

Handling

Culture Medium	DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)
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.
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.