Product sheet

HK-CRISPR-Nup93-mEGFP Cells | 300655

General information

Description	The HK-CRISPR-Nup93-mEGFP cell line is derived from Hela Kyoto cells and engineered using CRISPR/Cas9 technology to express Nup93 fused with monomeric enhanced green fluorescent protein (mEGFP). This allows for real-time visualization of Nup93 within the nuclear envelope, aiding in the study of nuclear-cytoplasmic transport, nuclear pore complex assembly, and nuclear envelope integrity. Nup93 is crucial for maintaining nuclear pore complex architecture and function. The mEGFP tag enables tracking its dynamics and interactions, facilitating high-resolution imaging techniques like confocal microscopy. This cell line helps researchers understand gene regulation, nucleocytoplasmic trafficking, and cellular stress responses. The HK-CRISPR-Nup93-mEGFP cell line is a valuable resource for studying cell biology and nuclear pore complex-associated diseases, contributing to potential therapeutic strategies targeting nuclear transport pathways. It is particularly useful for exploring the nuclear envelope's role in cellular function and pathology.
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-Nup93-mEGFP (Cytion catalog number 300655)
Biosafety level	1
NCBI_TaxID	9606

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Depositor	The Ellenberg Lab (EMBL)
GMO Status	GMO-S1: This HeLa Kyoto line contains an mEGFP knock-in at the endogenous Nup93 locus for nuclear pore structural studies. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Protein expression	Nup153, mEGFP-tag

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.
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.

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.