

HK-CRISPR-NUP205-mEGFP Cells | 301574**General information****Description**

The HK-CRISPR-NUP205-mEGFP cell line is a genetically engineered human cell line designed to study nucleoporin 205 (NUP205) and its role in the nuclear pore complex. Modified with CRISPR-Cas9 to tag NUP205 with monomeric enhanced green fluorescent protein (mEGFP), it enables visualization and tracking of NUP205 in live cells, aiding research into nuclear transport mechanisms and nuclear pore complex dynamics.

NUP205 is a critical nuclear pore complex component, regulating molecule transport between the nucleus and cytoplasm. Tagging NUP205 with mEGFP allows researchers to observe its localization and behavior in real-time under a fluorescence microscope, making this cell line particularly useful for studying nuclear pore complexes' structural and functional aspects and their roles in gene expression, RNA processing, and the cell cycle.

The HK-CRISPR-NUP205-mEGFP cell line is a powerful tool for investigating nucleocytoplasmic transport mechanisms and the nuclear pore complex's role in cellular homeostasis. It is also valuable for exploring how disruptions in nuclear pore function contribute to diseases like cancer and neurodegenerative disorders, offering a robust model for advancing our understanding of nuclear transport and its implications for human health.

Organism Human**Tissue** Endocervix**Disease** Adenocarcinoma**Synonyms** HK-CRISPR-NUP205-mEGFP #81**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-NUP205-mEGFP (Cytion catalog number 301574)

HK-CRISPR-NUP205-mEGFP Cells | 301574**Biosafety level** 1**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_UR49**Depositor** The Ellenberg Lab (EMBL)**GMO Status** GMO-S1: This HeLa Kyoto line contains a CRISPR-engineered mEGFP fusion at the NUP205 locus for scaffold-level nuclear pore research. 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 $300 \times g$ 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.