

HK-CRISPR-Tpr-mEGFP Cells | 300662

Renseignements généraux

Description

The HK-CRISPR-Tpr-mEGFP cell line is a specialized model developed for advanced genetic research, particularly in genome editing and gene expression studies. Derived from HeLa Kyoto cells, it integrates CRISPR/Cas9 technology for precise genomic modifications. The incorporation of the mEGFP (monomeric Enhanced Green Fluorescent Protein) reporter gene facilitates real-time visualization and tracking of cellular processes, making it a robust tool for studying gene function, protein localization, and dynamic cellular events in live cells.

This cell line is especially useful for nephrology research, drug discovery, and toxicology studies. The expression of the Tpr gene, a component of the nuclear pore complex, aids in understanding nuclear transport mechanisms and cellular compartmentalization. Researchers use HK-CRISPR-Tpr-mEGFP cells to explore the roles of nuclear pore proteins in various cellular pathways, contributing to insights in cancer, viral infections, and genetic disorders.

Organism Human**Tissue** Endocervix**Disease** Adenocarcinoma

Caractéristiques

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

Données réglementaires

Citation HK-CRISPR-Tpr-mEGFP (Cytion catalog number 300662)**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-tagged Tpr generated via CRISPR, enabling studies of nuclear basket architecture. This classification applies only within Germany and may differ elsewhere.

Données biomoléculaires

Protein expression Tpr, mEGFP-tag

Manipulation

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 $300 \times g$ 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.

Contrôle de la qualité et analyse moléculaire

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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.