

**HK-2xCRISPR-CAP-D2-mEGFP Cells | 301572****General information****Description**

The HK-2xCRISPR-CAP-D2-mEGFP cell line is a genetically modified HeLa Kyoto cell line. Engineered using CRISPR/Cas9 technology, these cells express the CAP-D2 protein fused with monomeric Enhanced Green Fluorescent Protein (mEGFP), enabling real-time visualization of CAP-D2 dynamics. The mEGFP marker allows researchers to study protein localization, trafficking, and interactions within the cells.

The genetic modifications provide insights into CAP-D2's role in cellular signaling, cytoskeletal organization, and stress responses. Additionally, the fluorescent marker enhances live-cell imaging and high-throughput screening, making this cell line essential for both basic and applied research.

**Organism** Human**Tissue** Endocervix**Disease** Adenocarcinoma**Synonyms** HK-2xCRISPR-CAP-D2-mEGFP #272-78, HK CRISPR CAP-D2-mEGFP**Characteristics****Age** 30 years**Gender** Female**Ethnicity** African American**Morphology** Epithelial-like cells with mosaic stone shape**Growth properties** Adherent**Regulatory Data****Citation** HK-2xCRISPR-CAP-D2-mEGFP (Cytion catalog number 301572)**Biosafety level** 1**NCBI\_TaxID** 9606**CellosaurusAccession** CVCL\_UR42

**HK-2xCRISPR-CAP-D2-mEGFP Cells | 301572****Depositor** The Ellenberg Lab (EMBL)**GMO Status** GMO-S1: This HeLa Kyoto line contains a CRISPR-engineered mEGFP knock-in at the CAP-D2 locus for condensin-complex studies. 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 NaHCO<sub>3</sub>, 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.

## HK-2xCRISPR-CAP-D2-mEGFP Cells | 301572

### 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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , 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  $-78^{\circ}\text{C}$  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  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

## HK-2xCRISPR-CAP-D2-mEGFP Cells | 301572

### **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.