

HK EGFP-Cap-D2 Cells | 300675

General information

Description

The HK EGFP-Cap-D2 cell line is an engineered variant of HeLa Kyoto cells, specifically designed for advanced research in cellular biology and genetic engineering. This cell line expresses enhanced green fluorescent protein (EGFP) fused to the C-terminus of the D2 dopamine receptor, enabling the visualization of receptor dynamics and distribution in real-time under fluorescence microscopy. This feature is particularly beneficial for studying receptor trafficking, signaling pathways, and the effects of pharmacological agents on D2 receptor behavior.

These cells are used extensively in neurological research to understand better the mechanisms underlying dopamine signaling, which is crucial in many neurological disorders such as Parkinson's disease, schizophrenia, and depression. The fusion of EGFP to the D2 receptor does not affect the receptor's normal function or its cellular localization, making HK EGFP-Cap-D2 a valuable tool for physiological and pathological studies. The stable expression of EGFP also allows for longitudinal studies in live cells, providing insights into the dynamic processes of receptor regulation and interaction with other cellular components.

Organism Human

Tissue Cervix

Disease Carcinoma

Synonyms HeLa Kyoto EGFP CAP-D2, HeLa Kyoto Cap-D2 EGFP

Characteristics

Age 30 years

Gender Female

Ethnicity African American

Morphology Epithelial-like cells with mosaic stone shape

Growth properties Monolayer, adherent

Regulatory Data

Citation HK EGFP-Cap-D2 (Cytion catalog number 300675)

Biosafety level 1

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NCBI_TaxID 9606

CellosaurusAccession CVCL_1D60

Depositor The Ellenberg Lab (EMBL)

GMO Status GMO-S1: This HeLa Kyoto line contains an EGFP-Cap-D2 construct enabling live-cell studies of condensin-II dynamics. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Protein expression EGFP-CAP-D2, About 80% of cells show expression: Location/Gene: 1..589 / Pcmv, 619..645 / Flag-tag, 646..660, 1375..1389/null, 661..1374 / EGFP, 1435..5638/CAP-D2, 6886..7680/KanR/NeoR

Products CMV Promotor, FLAG octapeptide, Glycin linker, Neomycin, Phosphotransferase

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.

Seeding density 1×10^4 cells/cm²

Fluid renewal 2 to 3 times per week

Post-Thaw Recovery After thawing, plate the cells at 5×10^4 cells/cm² and allow the cells to recover from the freezing process and to adhere for at least 24 hours.

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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.

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.

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.

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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

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.