HK Mad2-LAP/H2B-mCherry

300920

GENERAL INFORMATION

Description

The HK Mad2-LAP/H2B-mCherry cell line is a genetically engineered cell model extensively utilized for studying chromosome segregation and the spindle assembly checkpoint during mitosis. These cells are derived from HeLa Kyoto cells, a robust human cell line originally taken from a cervical carcinoma. The HK Mad2-LAP (LAP-tagged Mad2) aspect of the cell line facilitates the visualization and functional analysis of the Mad2 protein, a critical component of the spindle assembly checkpoint that prevents anaphase onset until all chromosomes are properly aligned at the metaphase plate.
Incorporation of H2B-mCherry, where histone H2B is tagged with the mCherry fluorescent protein, allows for real-time imaging of chromatin dynamics during cell division. This feature makes the HK Mad2-LAP/H2B-mCherry cell line an excellent tool for high-resolution live-cell imaging techniques to observe chromosomal movements and mitotic progression in human cells under various experimental conditions. The use of fluorescent tags aids in precise tracking and quantification, thereby providing valuable insights into the molecular mechanisms governing cell cycle regulation and chromosomal stability.

Tissue	Cervix
Disease	Carcinoma

Synonyms

- HeLa Kyoto Mad2-LAP and H2B-mCherry
- HeLa Kyoto Mad2-LAP

CLASSIFICATION

Organism	Human
Sex	Female
Growth properties	Adherent
GMO status	GMO-S1

GMO status details

GMO-S1: This HeLa Kyoto line contains Mad2-LAP and H2B-mCherry constructs enabling visualization of spindle checkpoint dynamics. This classification applies only within Germany and may differ elsewhere.

Biosafety level	1

CHARACTERISTICS

Morphology	Epithelial-like cells with mosaic stone shape

IDENTIFIERS

Citation

HK Mad2-LAP/H2B-mCherry (Cytion catalog number 300920)

NCBI TaxID	9606
Cellosaurus	CVCL_1D65
Depositor	The Ellenberg Lab (EMBL)

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EXPRESSION AND SUSCEPTIBILITY

Protein expression

Mad2-LAP/H2B-mCherry

HANDLING AND CULTURE

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)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

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 x 10^4 cells/cm^2
Fluid renewal	2 to 3 times per week
Post-thaw recovery	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hours.

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit. 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.
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.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
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.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

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.

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.

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.

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.

DONOR

Donor age	30 years
Ethnicity	African American

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Third-party agreements apply	Yes