HeLa mCherry-Histone H2B EGFP-Alpha Tubulin Cell Line
Catalog #152987

CancerTools.org
Global Research Tools Collaborative

Product description

The HeLa mCherry-Histone H2B EGFP-Alpha Tubulin cell line enables live-cell imaging of chromatin (both interphase and mitotic chromosomes) and microtubule dynamics. This allows for detailed study of mitosis, spindle dynamics, cytoskeletal organisation, and cellular responses to treatments.

Name: HeLa mCherry-Histone H2B EGFP-Alpha Tubulin cell line

Organism: Human

Tissue: Cervix

Disease: Cancer

Cancer Type: Cervical cancer

Cancers detailed: Cervical adenocarcinoma

Growth properties: Adherent

Production details: The human histone H2B gene was fused to the gene encoding mCherry and Alpha Tubulin was similarly fused to EGFP. Both constructs were transfected into human HeLa cells to generate a stable line constitutively expressing H2B-mCherry and EGFP-Alpha Tubulin. The mCherry-Histone H2B fusion protein was incorporated into nucleosomes without affecting cell cycle progression.

Model: Reporter line

Parental cell line: HeLa S3

Cellosaurus id: CVCL_HP86

Contributor(s)

Inventor: Francis Barr
Institute: University of Liverpool

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Recommended medium: DMEM + 10% Foetal Bovine Serum (FBS). Antibiotic selection for GFP and mCherry expression: 1 µg/mL Puromycin, 4 µg/mL Blasticidine. Expression is quite stable but selecting at least every two passages is recommended.

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Subculture: Split sub-confluent cultures (70-80%) 1:4 to 1:10 using Trypsin-EDTA solution; 5% CO2; 37°C. Please note that these cells tend to float and appear rounded.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimise exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension to a 50 mL conical tube using a pipette.
5. Rinse the vial with 1 mL of medium and add it dropwise to the cells, while gently swirling the 50 mL tube.
6. Wash by adding 15 - 20 mL of medium **dropwise**, while gently swirling the tube.
7. Centrifuge the cell suspension at **250 x g for 5 minutes** at room temperature.
8. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
9. Gently add required volume of culture medium and transfer to a suitable cell culture flask.

References

- Dunsch et al. 2012. J Cell Biol. 198(6):1039-54. PMID: 22965910.
- Zeng et al. 2010. J Cell Biol. 191(7):1315-32. PMID: 21187329.
- Bastos et al. 2010. J Cell Biol. 191(4):751-60. PMID: 21079244.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: HeLa mCherry-Histone H2B EGFP-Alpha Tubulin cell line, was invented by Francis Barr at the National Cancer Institute (CancerTools.org #152987).

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