Histone H2B-GFP expressing HeLa Cell Line

Cancer Cell Line

Cat. # SCC117

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

Data Sheet

Background

Chromatin, the higher order structure of DNA, proteins and RNA, constitutes the majority of the nucleus of eukaryotic cells. Changes in chromatin structure are the essence of many essential nuclear processes, including transcription, mitosis, meiosis, and apoptosis.[1] The nucleosome represents the primary building block of chromatin and it comprises an octomer of four core histone proteins (H2A, H2B, H3 and H4). HeLa cells expressing green fluorescent protein fused histone H2B (H2B-GFP) have been widely used to visualize the dynamics of chromosomal architecture in living cells during various processes.[2,3] In addition to studying normal mitosis, histone H2B-GFP has been utilized for imaging the distinctive clustering behavior of double minute chromosomes (DMs) in cancer cells during mitosis, which contributes to their asymmetric distribution to daughter cells.[2] Monitoring histone H2B-GFP also permits continuous analysis of chromosomal degradation during apoptosis.

Short Tandem Repeat (STR) Profile

D3S1358: 15, 18	D16S539: 9, 10
TH01: 7	CSF1PO: 9, 10
D21S11: 27, 28	Penta D: 8, 15
D18S51: 16	vWA: 16, 18
Penta E: 7, 17	D8S1179: 12, 13
D5S818: 11, 12	TPOX: 8, 12
D13S317: 12, 13.3	FGA: 21
D7S820: 8, 12	Amelogenin: X

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

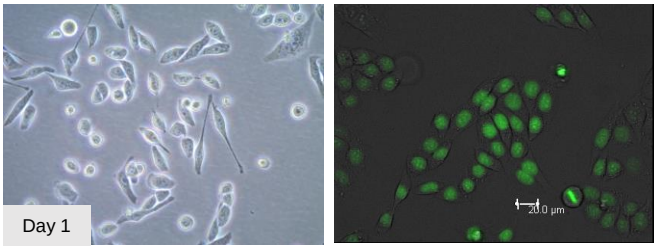
Storage and Handling

Histone H2B-GFP expressing HeLa Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥1X10^6 viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Data



References

1. Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev.Biochem. 67:545–579.

2. Kanda, T., et al. (1998) Histone–GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells. Curr. Biol. 8: 377-385.

3. Yamamoto, N., et al. (2004) Cellular dynamics visualized in live cells in vitro and in vivo by differential dual-color nuclear-cytoplasmic fluorescent-protein expression. Cancer Res. 64: 4251-4256.

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

   Cells are thawed and expanded in HeLa Expansion Medium containing DMEM-High Glucose Medium (EMD Millipore Cat. No. SLM-120-B), 2 μg/mL Blasticidin (EMD Millipore Cat. No. 203351-10ML), 10 μg/mL Ciprofloxacin HCL (Sigma Cat. No. PHR1044) and 10% FBS (EMD Millipore Cat. No. ES-009-B).
2. Remove the vial of frozen Histone H2B-GFP Hela cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of HeLa Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of medium all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of HeLa Expansion Medium.
10. Transfer the cell suspension to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10 -15 mL of fresh HeLa Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90-95% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the layer of 90-95% confluent Histone H2B-GFP HeLa cells.
2. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate at 37°C incubator for 3-5 minutes.
3. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
4. Add 8 mL of HeLa Expansion Medium to the plate.
5. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
7. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
8. Apply 2 mL of HeLa Expansion Medium to the conical tube and resuspend the cells thoroughly.
   Important: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:6 – 1:8).

Cryopreserving Cells

Histone H2B-GFP HeLa cells can be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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