FLAG-SNAP-hTERT expressing HeLa Cell Line

Immortalized Cell Line

Cat. # SCC112

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

Certificate of Analysis

Background

Telomerase play a critical role in regulating proliferation and senescence in normal somatic cells as well as most cancer cells. In humans, the telomerase reverse transcriptase (hTERT) is a lowly expressed protein with only several hundred molecules per cell. The low expression has made it difficult to detect, purify and study TERT activity from the endogenous loci.

To facilitate reliable detection of the endogenous TERT protein, a FLAG-SNAP tag was fused to the N-terminus of the TERT locus in HeLa cells. The FLAG-SNAP tag does not impair the function of the endogenous hTERT protein. Efficient immunopurification (IP) of the active telomerase ribonucleoprotein complexes may be accomplished by using the well-characterized FLAG antibody. The SNAP tag may be used to visualize subcellular localization of the low abundant endogenous TERT protein.

Short Tandem Repeat (STR) Profile

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

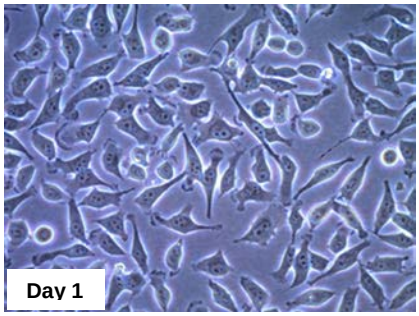
Storage and Handling

FLAG-SNAP-hTERT 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, Hepatitis A, B, C, and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- **Cells are positive for HPV-18 by PCR.**
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Representative Data



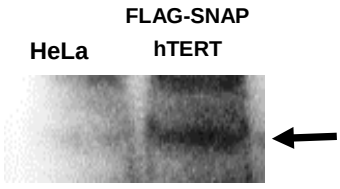


Figure 1. Day 1 after thawing (A). Western blot of cellular lysates of HeLa cells expressing FLAG-SNAP-hTERT using a FLAG antibody verify the expression of FLAG tagged TERT protein.

Reference

Xi L, Schmidt JC, Zaug AJ, Ascarrunz DR, and Cech TR (2016) A novel two-step genome editing strategy with CRISPR-Cas9 provides new insights into telomerase action and TERT gene expression. *Genome Biology* 16: 231-248.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

Thawing Cells

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

   Cells are thawed and expanded in DMEM-High Glucose (Sigma Cat. No. D6546), 10% FBS (EMD Millipore Cat. No. ES-009-B), 2 mM L-Glutamine (EMD Millipore Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C).

2. Remove the vial of frozen FLAG-SNAP-hTERT expressing 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 FLAG-SNAP-hTERT Expressing HeLa Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media 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 300 x 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 FLAG-SNAP-hTERT Expressing HeLa Expansion Medium.

10. Transfer the cell mixture 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 FLAG-SNAP-hTERT Expressing 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.

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Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of FLAG-SNAP-hTERT Expressing HeLa cells.

2. Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse.

3. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.

4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.

5. Add 8 mL of FLAG-SNAP-hTERT Expressing HeLa Expansion Medium to the plate.

6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.

7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.

8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.

9. Apply 2 mL of FLAG-SNAP-hTERT Expressing HeLa Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.

10. Count the number of cells using a hemocytometer.

11. Plate the cells to the desired density. Typical split ratio is 1:6 to 1:10.

Cryopreservation of Cells

FLAG-SNAP-hTERT Expressing HeLa Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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