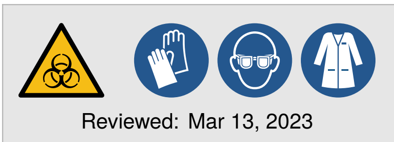
Cultivating HeLa cells



HeLa cells (ATCC No. CRM-CCL-2) are the first immortal human cells cultured in vitro and form the basis for countless discoveries in biology and medicine. They were derived in 1951 from a cervical adenocarcinoma biopsy taken from Henrietta Lacks at Johns Hopkins Hospital (Baltimore, MD, U.S.), without her knowledge or consent—a common practice at the time. Today, genomic and transcriptomic data from HeLa cells are under controlled access to protect the privacy of the Lacks family.

HeLa cells are hypertriploid with 70–145 chromosomes, including 22–25 clonally abnormal “HeLa signature” chromosomes. They are epithelial in origin and keratin-positive. The cell line expresses retinoblastoma protein (RB1) at normal levels but shows low expression of cellular tumor antigen p53 (TP53). HeLa cultures exhibit high heterogeneity in reproductive capacity. According to clonal lineage analysis (Sato et al., 2016), only 5% of the population is truly immortal.

HeLa S3 (ATCC No. CCL-2.2) is a clonal subline derived by Puck and Marcus (1956) that can grow in suspension culture (*Alternative S*). However, this growth mode alters anchorage-dependent signaling and metabolic responses.

This is a bench card. Full protocol available online.

Procedures

A › *Splitting adherent HeLa cells*

☐ R0081 0.06% Trypsin solution

(1.) Bring the growth medium, PBS, and trypsin to room temperature.

(2.) Aspirate off the growth medium and wash the cells once with PBS.

(3.) Cover the plate with 0.05% trypsin/EDTA and incubate at 37 °C for 2–3 min. — 2–3 min

(4.) Add an equal volume of growth medium containing serum, or 1 × trypsin inhibitor solution if applicable. Pipette up and down. Squirt the surface of the dish to detach all adherent cells.

> ***Critical:*** Remove at least 90% of the adherent cells to avoid selection bias in the culture.

(5.) *Optional:* Transfer the suspension to a conical tube. Centrifuge at 500 × *g* for 5 min. Resuspend the pellet in fresh growth medium. Count cells and assess viability.

(6.) Seed the cells at the desired density in a new culture dish. Spread by rocking the dish back and forth.

> ***Critical:*** Do not swirl in a circular motion—this causes the cells to clump in the center of the dish.

F › *Freezing HeLa cells for long-term storage*

☐ Cryovial, polypropylene, non-pyrogenic, with seal rings ☐ Dimethyl sulfoxide

(1.) Prepare cryo-preservation medium by supplementing DMEM with 20% FBS and 10% DMSO.

(2.) Bring the cryo-preservation medium, PBS, and trypsin to room temperature.

(3.) Detach cells from a confluent 10 cm dish using standard trypsinization.

(4.) Collect the cells by centrifugation at 500 × *g* for 3 min. Carefully aspirate the supernatant.

(5.) Resuspend the pellet in 2 mL cryo-preservation medium to a final concentration of about 4.0×10^{6} mL^{-1}.

(6.) Aliquot 1 mL per vial into two cryovials. Label with water-resistant marker or deep-freeze label.

(7.) Slow-freeze the vials at −80 °C overnight using a styrofoam container or freezing device. Transfer to liquid nitrogen storage the next day.

T › *Thawing a cryo-preserved HeLa cell line*

(1.) Warm 20 mL of growth medium to 37 °C; aliquot 10 mL each into two conical tubes.

(2.) *Critical:* Quickly thaw the cryogenic vial in a 37 °C water bath.

> ***Critical:*** Ensure the lid is tightly sealed and keep the vial upright to avoid contamination. Wipe thoroughly with disinfectant before transferring to a sterile environment.

(3.) Transfer the thawed cell suspension into one of the conical tubes with pre-warmed growth medium.

(4.) Centrifuge at 500 × *g* for 3 min. Carefully aspirate the supernatant to remove DMSO.

(5.) Resuspend the cell pellet in the second tube containing 10 mL pre-warmed growth medium.

(6.) Plate the resuspended cells onto a 10 cm culture dish.

(7.) Incubate at 37 °C under 5% CO_2. Once attached and recovered, passage cells for the first time (P1).

S › *Bringing and maintaining HeLa S3 in suspension culture*

☐ Magnetic spinner flask	☐ Serum-free medium (EX-CELL® Medium)

(1.) Over the course of one to two weeks, gradually reduce the FBS content in the culture medium to 2%.

(2.) Dissociate the cells using standard trypsinization.

(3.) Plate the cells for 48 h in serum-free medium (without L-glutamine) such as EX-CELL® Medium.

(4.) Dissociate the cells again. Count and assess viability.

(5.) Seed 5.0×10^5 mL^{-1} viable cells into 3 magnetic spinner flasks. Stir gently at 40–70 rpm overnight.

(6.) After 24 h, collect cells from the medium. Count and assess viability.

> ***Critical:*** If viability is below 50%, use a Ficoll® gradient to remove dead cells by centrifugation.

(7.) Pool suspension cells from two or three flasks into a new spinner flask with fresh medium. Inoculate at no more than 2.5×10^5 mL^{-1}.

(8.) Continue culture for three days with gentle stirring. Then harvest by centrifugation and check viability.

> ***Critical:*** Do not disturb cells that have adhered to the spinner walls.

CC-BY-SA-4.0

List of references

T. Puck and P. Marcus, *J. Exp. Med.* **103**(5), 653—666 (1956).

S. Sato, A. Rancourt, Y. Sato, and M.S. Satoh, *Sci. Rep.* **6** 23328 (2016).

Recipe (available online) Troubleshooting (available online) Notes (available online)

Lab Protocols — © B. C. Buchmuller. Licensed CC-BY-SA-4.0. Research use; provided as-is, without warranty. Current when printed. Visit https://benjbuch.github.io/check/?q=2cc6bcd or scan the QR code to read the full protocol or to check for updates.