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 70–164 chromosomes, including about 20 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 3–6% of the population is truly immortal.

HeLa S3 (ATCC No. CCL-2.2) is a clonal subline derived by Puck and Marcus (1955); Puck et al. (1956) that can grow in suspension culture (*Alternative S*). However, this growth mode alters anchorage-dependent signaling and metabolic responses.

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

□ Dispose waste after inactivation as REGULATED MEDICAL WASTE



Reviewed: Oct 26, 2026

Briefly

○ For routine maintenance of adherent cells:

- Grow in DMEM (with 4 mM glutamine) + 10% FBS, at 37 °C under 5% CO_2 in a humidified atmosphere.
- *Optional:* Supplement 50–100 U/mL penicillin + 50–100 µg/mL streptomycin.

  Quality assurance: Avoid antibiotics and antimycotics for routine culture work. They may mask contamination and interfere with metabolism in sensitive cells.
- Subculture at 70–80% confluence by passaging 1:5 every 48–72 h.

  Quality assurance: Test cultures regularly for mycoplasma contamination.

  Critical: Discard cultures after about 50 passages. HeLa cells do not show contact inhibition. Over-confluent cultures should be discarded to maintain a homogenous growth pattern and metabolic state.

○ For suspension HeLa S3 cells:

- Grow in EX-CELL® or equivalent serum-free medium, stirred at 40–70 rpm, at 37 °C as above.
- Subculture at 2.5×10^5 mL^{-1} every three days.

Procedures

A › *Splitting adherent HeLa cells*

- ☐ PBS, pH 7.5 (at 1×) ◇ R0037
- ☐ HeLa growth medium
- ☐ 0.05% Trypsin solution ◇ R0081
- ☐ 0.25% Trypsin inhibitor solution (*optional*) ◇ R0082

(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 0.4 volumes of the 0.25% trypsin inhibitor stock if applicable. Pipette up and down. Squirt the surface of the dish to detach all adherent cells.

(5.) *Optional:* Transfer the suspension to a conical tube. Centrifuge at 200 × *g* for 10 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*

- ☐ DMSO
- ☐ 0.05% Trypsin solution ◇ R0081
- ☐ Cryovial, polypropylene, non-pyrogenic, with seal rings
- ☐ Controlled-rate freezing device

(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 200 × *g* for 10 min. Carefully aspirate the supernatant.

(5.) Resuspend the pellet in 2 mL cryo-preservation medium to a final concentration of about 2.0×10^{6} mL^{-1}.

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

> ***Note:*** One million cells cover about 60% of a 10 cm dish.

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

> ***Hint:*** A basic styrofoam box is sufficient for HeLa cells, but freezing by using Mr. Frosty™ will improve viability.

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 200 × *g* for 10 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).

> ***Hint:*** Conduct experiments from passage P3 onwards for best consistency.

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 $5.0\times10^{5}\,mL^{-1}$ 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.

Hint: Some cells may adhere to the spinner walls — ignore them. Dead cells in suspension are common at this stage.

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 $2.5\times10^{5}\,mL^{-1}$.

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

Quality assurance: Viability should reach 85–90% by the second round.

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

Hint: Expect stabilization and improved growth after two to three weeks of adaptation.

Troubleshooting

Splitting adherent HeLa cells

In Step 3:

– Cells clump after trypsinization
  - Ensure trypsin covers the entire plate surface. Increase incubation time for strongly adherent cultures.
  - Pipette more vigorously to break up clumps. Pass through a cell strainer if needed for counting accuracy.

Freezing HeLa cells for long-term storage

In Step 7:

– Low viability after thawing frozen stocks
  - Freeze cells at high density (above 1×10^{6}/mL) in log phase. Stationary-phase cells survive cryopreservation poorly.
  - Ensure controlled-rate freezing. Direct placement at −80 °C without insulation can cause ice crystal damage.

Thawing a cryo-preserved HeLa cell line

In Step 7:

– Low viability after thawing
  - Ensure rapid thawing in a 37 °C water bath.
  - Minimize time the cells are exposed to DMSO before dilution. Immediately remove DMSO by centrifugation.
  - Do not disturb the cells during the initial recovery phase. Avoid replating at very low density.

– Cells fail to attach
  - Make sure cryoprotectant (DMSO) is fully removed.
  - Use tissue-culture treated dishes.
  - Allow 24–48 h for cells to recover before first passage.

Materials

Splitting adherent HeLa cells

- ☐ **Phosphate-buffered saline (PBS)**, pH 7.5 (at 1 ×) ⊘ R0037
- ☐ **HeLa growth medium**
  Store at 4 °C.
- ☐ **Trypsin solution**, 0.05% ⊘ R0081
- ☐ **Trypsin inhibitor solution**, 0.25% ⊘ R0082 (*optional*)

Freezing HeLa cells for long-term storage

- ☐ **Dimethyl sulfoxide (DMSO)**
- ☐ **Trypsin solution**, 0.05% ⊘ R0081
- ☐ **Cryovial**, polypropylene, non-pyrogenic, with seal rings
- ☐ **Controlled-rate freezing device**
  - *e.g.* **Mr. Frosty™ Freezing Container**
    Thermo Fisher Scientific

Bringing and maintaining HeLa S3 in suspension culture

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

Recipes

HeLa growth medium

Amount	Ingredient	Stock	Final
500 mL	DMEM		
50 mL	Fetal bovine serum (FBS)		10%

Use sterile liquids. Store at 4 °C.

HeLa growth medium



Contains a component outside the substance collection; classify it from its SDS before use.

☐ Hold for EHS review before disposal

Date: Sign:

List of references

American Type Culture Collection, *HeLa* (*CCL-2*) (American Type Culture Collection, 2026). (Product sheet CCL-2)

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T.T. Puck and P.I. Marcus, *Proc. Natl. Acad. Sci. U.S.A.* **41**(7), 432-437 (1955).

T.T. Puck, P.I. Marcus, and S.J. Cieciura, *J. Exp. Med.* **103**(2), 273-283 (1956).

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

Change log

2023-03-13 **Elisabeth Gardiner, David Altman, Benjamin C. Buchmuller** Version 1, 2012 to 2023. The HeLa S3 suspension protocol of The Scripps Research Institute, released on ResearchGate; published in part as Woods Hole Physiology Course 2006 (doi:10.1575/1912/8720). Adapted as an SOP in 2023.

2026-10-26 **Benjamin C. Buchmuller** Version 2. Credited ATCC (CCL-2), Puck et al. (1955, 1956), Macville et al. (1999), the Woods Hole HeLa protocols, and Elisabeth Gardiner (Scripps) for the suspension culture; corrected the centrifugation to 200 × g, the karyotype range, the glutamine supplement, the freezing density, the passage limit, and the trypsin inhibitor quench.

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