Cultivating HeLa cells

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



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This is a bench card. Full protocol available online.

A › *Splitting adherent HeLa cells*

(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*

(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 $2.0\times10^{6}\ \mathrm{mL}^{-1}$.

(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 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).

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

(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 2.5×10^5 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.

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

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