

Essential Cell Culture Protocol

A focused guide on seeding, maintaining, freezing, and protecting your cultures

Seeding Cells: Starting Your Culture

1. Thaw cryopreserved vial in a 37°C water bath (1–2 min)
2. Dilute in 10 mL warm media
3. Centrifuge at 300g for 5 min
4. Discard supernatant, gently resuspend pellet
5. Seed cells at ~30–40% confluency

How much to seed? Example for HeLa Cells

Vessel	Area (cm ²)	Media Volume	Cells at 100% Confluency
6-well plate	~9.5	1–3 mL	0.3–1.2 × 10 ⁶
12-well plate	~3.8	1–2 mL	0.1–0.5 × 10 ⁶
24-well plate	~2.0	0.5–1 mL	0.05–0.24 × 10 ⁶
96-well plate	~0.3	0.1–0.2 mL	0.01–0.04 × 10 ⁶
T25 flask	25	3–5 mL	~0.7–2.8 × 10 ⁶
T75 flask	75	5–15 mL	~2.1–8.4 × 10 ⁶

- Remove DMSO quickly – it's toxic
- Leave a small piece of ice in the vial to ensure a gentle thaw.
- Work fast but gentle to reduce cell stress.
- Seeding density depends on the experimental setup.

Maintaining Healthy Cultures

1. Change media every 2–3 days or when yellow
2. Passage cells at 70–90% confluency
 - 2.1. Remove media and wash with PBS (5–10 ml)
 - 2.2. Add 2–3 mL trypsin, incubate at 37°C for 3–7 min, and tap to detach cells.
 - 2.3. Add complete media (10–20mL) to neutralize trypsin and resuspend cells.
 - 2.4. Centrifuge at 300g for 5 min, discard supernatant, and resuspend in appropriate level of media.
3. Label everything with passage number and date
4. Assessing your culture with SnapCyte: Analyze microscope images to measure confluency, count cells, and track doubling time.

- Warm media to 37°C
- Don't overfeed; top up if needed
- Check cells daily
- Flag unusual growth early
- Label everything with passage number, cell number and date
- Freeze slowly using a controlled rate (~1°C/min)

Freezing Cells: Long-Term Storage

1. Harvest cells at 70–80% confluency
2. Count and resuspend in freezing media (10% DMSO + 90% FBS)
3. Aliquot into cryovials (~1–5 million cells/vial)
4. Transfer to liquid nitrogen for long-term storage