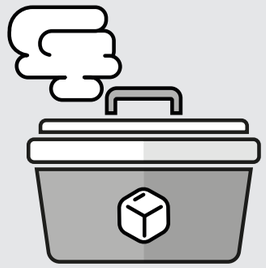
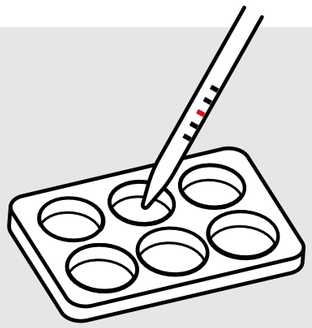
Best practices for primary cell culture

Follow these steps to ensure healthy and reproducible cell cultures*



Transportation

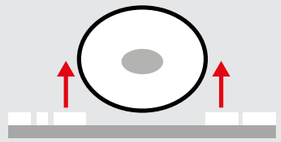
- After removal from liquid nitrogen, immediately place the cryovial in dry ice even for short distances, i.e., between storage and water bath.



Laminar flow cabinet

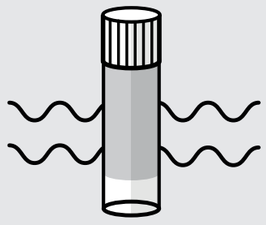
- Once the content is thawed, work swiftly. Disinfect the vial with 70% EtOH by rinsing with a squeeze bottle. Spraying the vial is not sufficient.
- Gently pipette the thawed cell suspension up and down 1–2 times. Then dilute the cells as quickly as possible by directly plating them in sufficient growth medium to give a DMSO concentration <1%. Alternatively, you can dilute the thawed cells 1:10 with medium, remove the DMSO by centrifugation, and then seed the cells in growth medium.
- The optimal size of the tissue culture vessel can be calculated based on the cell count and recommended seeding density, which can be found on the cell lot Certificate of Analysis, and cell manual, respectively. Ensure Cell Growth Medium has all components (basal medium + supplements) and is within suggested shelf-life timeframe.
- Change the medium the next day and every 2–3 days after that.
- Subculture when the cells have reached 70–90% confluency.

Enzymatic digestion



- Do not trypsinize the cells longer than necessary. For most cell types, we recommend detaching the cells at room temperature. Extended trypsinization times may lead to irreversible damage.
- Use Trypsin Neutralization Solution (TNS) to stop the trypsin reaction. Serum-reduced or serum-free media will not sufficiently inactivate the trypsin. If using Cell Growth Medium, make sure the serum concentration is >10% and the cells tolerate serum (some cell types stop growing when they come into contact with serum).

Thawing — 1 — 2 — 3 — 4 — Subculture — 5 — 6

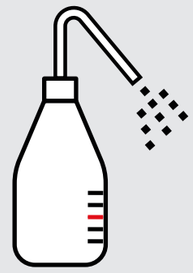


Water bath

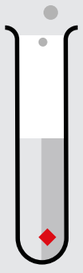
- Use a 37°C water bath. Do not thaw the vial in the incubator or in your hands. Before immersing the vial into the water up to the cap, make sure the cap of the vial is tightly closed. Set a 2-minute timer and only remove the vial from the water after 2 minutes. Do not repeatedly insert and remove the vial while thawing in water. If the content is still frozen, continue for ~15–20 more seconds.
- Do not use a floater. Ensure one vial is completely thawed, from water bath to plating, before thawing another vial.
- If you have a bead bath, thaw the vial in a beaker of water in the bead bath. Ensure the water is heated to 37°C.

Sterility

- Use aseptic working techniques during all cell culture work. Contaminations can be introduced into the culture from the water bath and when unsterile objects come into contact with open tissue culture vessels or media bottles.
- Clean and disinfect the laminar flow cabinet, incubator, and water bath regularly.



Cell count



- Count the cells after detachment to determine the optimal split ratio (based on the recommended plating density and the number of cells). Keep a note of the number of population doublings that occurred during the passage.
- Spin down the cells to remove the trypsin/EDTA and stop solution. Transferring EDTA into the new culture can lead to slower growth and may even halt growth.

*This best practices guide does not replace the product manuals. Always read carefully each product specific protocol and follow the instructions to ensure a successful experiment.

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