

Thawing cells protocol

1. Remove the cryovial containing the frozen cells from liquid nitrogen storage and **immediately** place it into a 37°C water bath.
2. Quickly thaw the cells (< 1 minute) by gently swirling the vial in the 37°C water bath until there is just a small bit of ice left in the vial.
3. Transfer the vial into a laminar flow hood. Before opening, wipe the outside of the vial with 70% ethanol.
4. Transfer 1 ml of thawed cells **dropwise** into the centrifuge tube containing 9 ml (HEK/CHO), 4 ml (HPC7/WEHI) of pre-warmed complete growth medium (DMEM-HEK293, WEHI, RPMI-CHO, IMDM-HPC7)
5. Centrifuge the cell suspension at approximately 200 × g for 5 minutes (HEK, WEHI, CHO) 200g for 4 min (HPC7).
6. After the centrifugation, check the clarity of supernatant and visibility of a complete pellet. Aseptically decant the supernatant without disturbing the cell pellet.
7. Gently resuspend the cells in complete growth medium, and transfer them into the appropriate culture vessel (10/5 ml plate) .