



## THAWING CRYOPRESERVED CELLS

All cryovials should be stored in liquid nitrogen immediately upon arrival, unless thawed for cell culture.

### Protocol

1. Remove the cryovial from the liquid nitrogen storage tank.
2. Thaw the cells quickly by placing the lower half of the vial into a 37°C water bath while agitating gently, remove after 60 seconds. Keep the cap out of the water to avoid contamination. There should still be a few ice crystals left after thawing (it is important not to over-thaw the cryovials as the presence of DMSO is toxic to the cells).
3. Decontaminate the vial by spraying and wiping the exterior of the vial with 70% ethanol. From this point onwards, all operations should be strictly carried out inside a laminar flow hood in aseptic conditions.
4. Gently re-suspend the cells in the vial and transfer the cell suspension into a 15 mL sterile conical tube containing 5 mL of pre-warmed, complete media using a sterile transfer pipette.
5. Centrifuge the cells at 1500 rpm for 3 minutes to pellet (the actual centrifuge duration and speed may vary, depending on the cell type).
6. Check the clarity of the supernatant to ensure that all cells are pulled down into the pellet. Aspirate out the supernatant without disturbing the cell pellet.
7. Re-suspend the cell pellet in fresh, pre-warmed culture media and transfer the cells into a culture vessel. Gently rock the culture vessel to distribute the cells evenly. Table 1 provides guidelines to the volume of culture media needed for a range of culture vessels.
8. Incubate the culture at 37°C, 5% CO<sub>2</sub>, or another recommended culture environment specified by the cell line datasheet. Incubate for at least 24 hours before processing the cells for downstream experiments.

**Table 1: Volume of Culture Media for Different Culture Vessels**

Culture Wares	Area (cm <sup>2</sup> )	Volume
12- well plate	1.12	2.0 ml/ well
6- well plate	4.67	3.0 ml/ well
T-25 flask	25	7.0 ml/ flask
T-75 flask	75	20.0 ml/ flask
100 mm dish	78	10.0 ml/ dish
150 mm dish	176	20.0 ml/ dish

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