General Guidelines For Thawing Cryopreserved Cells

All cryovials should be stored in liquid nitrogen immediately upon arrival, unless thawed for cell culture. **We strongly recommend end user to follow all proper instructions before working with the product. Refer to cell line specific instructions on the product page. These are the general guidelines for thawing cryopreserved cells.**

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 biological safety cabinet 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 200x g for 3 minutes to pellet. Aspirate out the supernatant without disturbing the cell pellet.
6. Re-suspend the cell pellet in fresh, pre-warmed culture media and perform a viable cell count.
7. Transfer the cells into a culture vessel using the recommended seeding density. Gently rock the culture vessel to distribute the cells evenly. Table 1 provides general guidelines to the volume of culture media needed for a range of culture vessels.

   Note: *The size of the culture vessel is subjected to the seeding density of the cell line (available on the corresponding cell line webpage "seeding density" section). Please use the viable cell count from step 6 and seed the viable cells following the "seeding density" specified on the corresponding cell line page. Otherwise, we recommend thawing entire cryopreserved content into T25 flask with instructed medium condition from the propagation section of the product page.*
8. Incubate the culture at 37°C, 5% CO2, or another recommended culture environment for the specific cell line. Incubate for at least 24 hours before processing the cells for downstream experiments.

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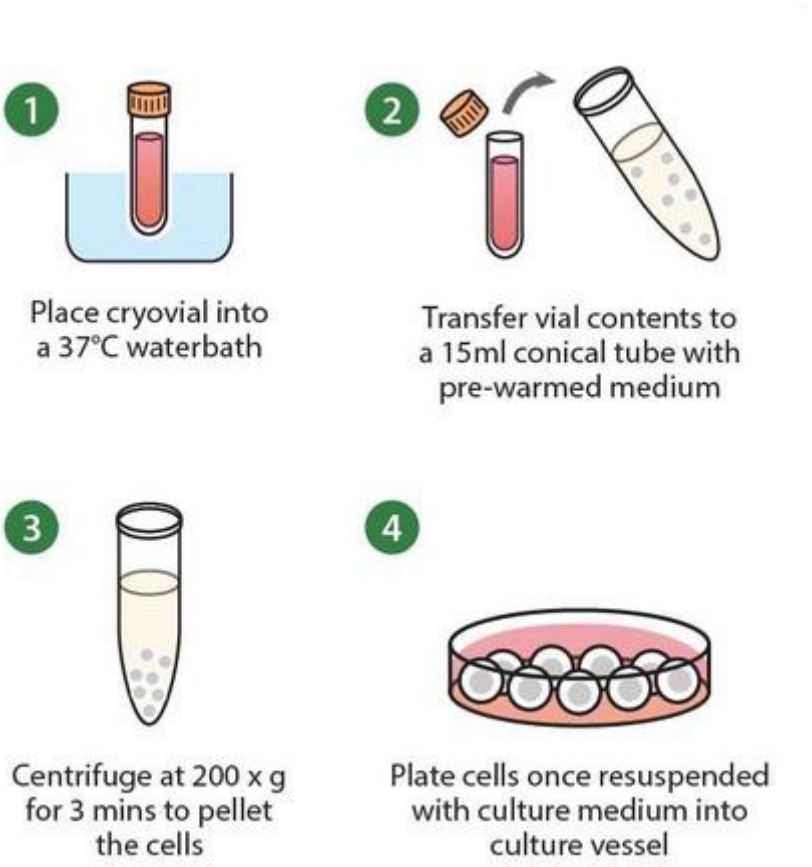

Table 1: Volume of Culture Media for Different Culture Vessels

Culture Wares	Area (cm^2)	Volume
12- well plate	4	2.0 ml/ well
6- well plate	9	3.0 ml/ well
T-25 flask	25	7.0 ml/ flask
T-75 flask	75	20.0 ml/ flask
100 mm dish	55	10.0 ml/ dish
150 mm dish	152	20.0 ml/ dish

For laboratory research only. Not for clinical applications.

For technical questions, please email us at technical@abmgood.com

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