

Thawing of Cryopreserved Samples

iQ Biosciences performs the following thawing procedure when using cryopreserved samples to ensure optimal viability and cell recovery after thaw.

Materials & Equipment Required:

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|--|--------------------------|
| Water Bath | Conical centrifuge tubes |
| Refrigerated centrifuge Biosafety hood (Class II) | 70% ethanol |
| Microscope | Dry Chemwipes |
| Hemocytometer | Trypan Blue Solution |

(Optional: add 0.1 mg/mL DNase I to tissue culture media if large cell numbers ($>100 \times 10^6$) are being thawed. However, it is recommended not to use DNase if cells are to be used for purification of genomic DNA, for cDNA/RNA synthesis, or if cells are to be used in methycellulose-based media.)

Procedure

1. iQ Biosciences' cryopreserved vials contain ~1.0 to 1.8mL of cells in cryosolution depending on number of cells cryopreserved.
2. Aliquot 5 mL of complete tissue culture media (e.g. RPMI-1640 with 10%FCS) into a sterile 15 mL conical tube.
3. Spray the cryovial sample with 70% ethanol and dry using a ChemWipe.
4. In biosafety hood, twist the cap a quarter-turn to relieve pressure and then retighten the cap.
5. In a 37°C water bath, quickly thaw the vial by moving the vial in the water while not submerging the entire vial in the water bath. Visually observe and do not remove the vial until a tiny ice-crystal is left.
6. Remove vial from water bath.
7. Spray the cryovial sample with 70% ethanol, and dry using a ChemWipe.
8. In biosafety hood, transfer cells from the cryovial to the conical containing 5 mL of media of interest.
9. Rinse the inside of the cryovial with media from the conical tube and transfer back into the conical tube.
10. Centrifuge the conical tube at 300 x g for 10 minutes with brake on and remove the supernatant.
11. Resuspend cell pellet in appropriate tissue culture media and volume:
 - a. For 1-10 million unit, resuspend to 1-4mL of media to reach an approximate cell concentration of 1-2.5 million cells per mL.
 - i. For automatic counting, we recommend a 1:2 dilution with trypan blue
 - ii. For manual counting, we recommend a 1:10 dilution.
 - A. Apply this formula for manual counts calculation:
$$\text{Cell count} = (\text{Average cell count per gridded square}) * (\text{Dilution Factor}) * (10^4) * (\text{Volume})$$
 - a. For 20-100 million unit, resuspend to 1-5mL of media to reach an approximate cell concentration of 20 million cells per mL.
 - i. For automatic counting we recommend a 1:10 dilution with trypan blue
 - ii. For manual counting we recommend a 1:100 dilution with trypan blue
 - A. Apply this formula for manual counts calculation:
$$\text{Cell count} = (\text{Average cell count per gridded square}) * (\text{Dilution Factor}) * (10^4) * (\text{Volume})$$
12. Perform cell count and viability by trypan blue and examine the cells on a hemocytometer.
(www.hauserscientific.com/products/hausser_bright_line.html)

Reference

G-force calculation formula

$$G\text{-force} = 1.12 \times \text{Radius} \times (\text{rpm}/1000)^2$$