Cryopreserved Leukopak Thawing Protocol

ALLCELLS CRYOPRESERVED LEUKOPAK THAWING GUIDE

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GENERAL INSTRUCTIONS

1. Recommended thawing media for diluting and washing cells are RPMI 1640, IMDM, or DMEM supplemented with 10% heat inactivated FBS warmed to room temperature (20-25°C).
2. Proper aseptic technique should be used when handling and manipulating cells.
3. Cryopreserved products should be counted immediately after thawing before any further manipulation. AllCells is not liable for any cell loss during subsequent processing or manipulation.

THAWING INSTRUCTIONS

4. Place thawing media in a 37°C water bath, spray with 70% ethanol and place in the BioSafety Cabinet.
5. Remove cryobag from storage and place it in a 37°C water bath immediately. Make sure the bag is completely submerged and stable with no movement. During the thaw, you may check the status by gently inverting the bag and placing back into the water bath.
6. When the majority (approximately 90%) of the contents are thawed, place the bag on the benchtop at room temperature to complete thaw.
7. Spray or wipe the bag with 70% ethanol and transfer to the BioSafety Cabinet for further manipulation. You may ensure proper suspension by gently inverting the bag two times.
8. Using the port, gently empty the contents of the bag into a sterile 100ml conical tube. You can add an additional 5-10 ml of thawing media to the empty bag to rinse it off into the tube.
9. Slowly add thawing media to the tube containing the cells up to 4 times the volume of the cell suspension. Securely cap the tube and gently invert 3-5 times after adding all the media. Remove a sample to assess cell count and viability.
10. Centrifuge the tube at 300g for 20 minutes at 4°C (no brake). Make sure there is a cell pellet at the bottom of the tube at the end of the run.
11. Aspirate the supernatant slowly to avoid dislodging the cell pellet. Discard the supernatant.
12. Resuspend the cell pellet* by slowly adding in the appropriate cell media to the desired volume to establish the needed cell concentration. Confirm cell count and viability.

    * Make sure the cell pellet is properly dislodged and mixed. You may flick the cell pellet prior to adding the media.