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Protocol – Thawing Cryopreserved **Leukopak**

Materials

10, 25mL Serological pipettes
50 mL conical tube(s)
Sterile Transfer pipettes
Pipette aid
Swinging-bucket centrifuge
Laminar Flow hood
37°C water bath
Sterile scissors
Microscope

Reagents

Complete growth medium
EDTA Solution, sterile (optional)
DNase I Solution (optional)
Trypan blue (optional)

Storage Recommendation:

Cryopreserved leukopak should be stored in the "vapor phase" of a liquid nitrogen tank.

Thawing Procedure:

1. In a 37°C water bath, warm appropriate growth medium (e.g 10% FBS/90%RPMI), spray with 70% alcohol, and keep in hood.
2. Remove the cryopreserved leukopak from long term storage.
3. In a 37°C water bath, submerge the leukopak under without movement .
4. Once the leukopak has thawed to the point that there is only a sliver of ice remaining, remove the leukopak from the bath.
5. Clean the outside of the bag with 70% alcohol to prevent contamination.
6. Using sterile scissors, cut the port and transfer leukopak to sterile 50mL conical tubes, filling 1/3.
7. Dropwise, add an equal volume of growth media to the conical tubes.
8. Rinse the bag with an appropriate volume of growth media and gently add to conical tube.
9. Slowly bring up the volume in the conical tube by adding medium dropwise so that the conical tubes are filled.
10. Centrifuge the cell suspension at 300 x g at room temperature for 15 minutes.
11. Using a pipette, carefully remove most of the supernatant and save in a separate conical tube. Leave a small amount of supernatant behind so the cell pellet is not disturbed . ***Note:*** *DMSO is heavier than medium. Try to aspirate and discard the supernatant soon after centrifugation.*
12. While gently shaking the tube, slowly add an additional 15 to 20 mL of fresh medium to the tube and resuspend the pellet to a uniform suspension.
13. Centrifuge the cell suspension at 300 x *g* at room temperature for 15 minutes.
14. Using a pipette, carefully remove most of the supernatant. Leave a small amount of supernatant behind so the cell pellet is not disturbed. Gently resuspend the cell pellet in an appropriate amount of growth media to count cells.
15. Count the cells and determine viability using the Trypan Blue Method or other preferred methods for cell concentration and viability assessment.
16. If the cell count is lower than expected, centrifuge the supernatant saved in Step 12 at a slightly higher speed, count and combine if necessary.
17. Your cells are now ready for downstream applications.

References

1. Ramachandran *et al.* Optimal Thawing of Cryopreserved Peripheral Blood Mononuclear Cells for Use in High-Throughput Human Immune Monitoring Studies *Cells* 2012, 1, 313-324
2. Hemacytometer Counting Tool: http://www.currentprotocols.com/WileyCDA/CurPro3Tool/toolId-10.htmL

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Frequently Asked Questions

My Leukopak PBMCs are clumping, what can I do?
To prevent cell-cell interactions, the initial wash solution may be modified to consist of HBSS (without calcium and magnesium), 10% FBS and 2mM EDTA. If there appear to be stringy fragments in the solution, it may be due to dying cells which release DNA into the media. DNase I, added at final concentration of 0.1 mg/mL (or 200 Kunitz units/mL) and incubated at room temperature for 15 minutes, will reduce the tendency for cells to stick together.

My cells are too dilute, what should I do?
Some cells prefer to be in close contact with each other in culture. The appropriate plate or flask size will vary depending on the number of cells frozen in the leukopak. It is recommended that thawed cells be plated at a high density to optimize recovery. If necessary, try transferring the culture to a smaller flask until the cell density increases.

The viability after thawing is low, why?
The freezing and thawing process is stressful to most cells. Be sure to handle the cells very gently. Do not vortex, bang the vial to dislodge the cells, or centrifuge the cells at high speeds. In addition, decrease the time required to thaw the cells as prolonged exposure to cryopreservative can be toxic to the cells.

FAQs can also be found at http://www.bioivt.com/faq