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Thawing Instructions for Discovery Cryo Leukopaks™ (RUO)

Materials Needed:
HBSS (w/o calcium or magnesium) + 10% FBS + 0.1 mg/ml DNase (Thawing Buffer)
** We recommend the use of DNase to prevent cell clumping due to DNA leakage from dead cells.**
70% Ethanol
Discovery Cryo Leukopak (RUO)
Sterile bottle
Sterile scissors
Disposable pipettes and micropipette tips

Equipment:
Biological Safety Cabinet
Water Bath at 37C
Serological and Micropipettes
Liquid Nitrogen Freezer
Automated Cell Counter

Protocol

1. Warm HBSS + 10% FBS in 37C water bath. Wipe with 70% ethanol and transfer into the biological safety cabinet.
2. Add DNase to 0.1 mg/ml final concentration.
3. Remove Discovery Cryo Leukopak (RUO) from liquid nitrogen storage. Immediately place in a 37C water bath and fully submerge. Do not move the leukopak while it is thawing.
4. Once only a small ice crystal remains, remove the leukopak from the water bath, thoroughly clean the outside of the bag with 70% ethanol, and transfer into the biological safety cabinet.
5. Using sterile scissors, cut the port on the leukopak and slowly transfer the cell suspension into an appropriate sterile bottle.

6. Add an equal volume of Thawing Buffer to cell suspension dropwise.
7. Add one-half volume of Thawing Buffer to the original leukopak, thoroughly mix, and transfer to cell suspension.
8. Gently add an additional two volumes of Thawing Buffer to the cell suspension.
9. Thoroughly mix the sample, and take a small sample for cell count and viability.
10. Samples are ready to be centrifuged and downstream analyses.