Thawing protocol for cryopreserved leukopaks

BioSupplies
Biological material solutions

Cell Therapy

Leukopaks

MATERIALS



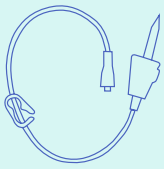
Cryopreserved leukopaks



Wash buffer (PBS pH 7.2, supplemented with 1 mM EDTA and 0.5% HSA)



Cultivation medium



Universal spike adapter with Luer Lock



50-mL syringe with Luer Lock



Dry or water bath

THAWING PROCEDURE

1. Pre-heat the bath to 37 °C.
2. Pre-heat wash buffer to 37 °C.
3. Retrieve the cassette with the frozen leukapheresis concentrate from nitrogen storage and transport it on dry ice.
4. Incubate the cassette for about 10 seconds at 37 °C before opening the cassette to remove the cryobag.
5. Incubate the cryobag for 3 minutes at 37 °C. The leukapheresis concentrate should still contain small ice crystals. Note: Cryopreserved leukopaks contain DMSO. Thawed cells should be processed immediately.
6. Once thawed, remove the leukopak from the bath and clean the bag to avoid contamination.
7. Carefully spike the cryobag with the spike adapter with Luer Lock connection (remove one of the spike ports for this).
8. Attach a syringe with 50-mL pre-warmed wash buffer to the Luer connection and slowly add the wash buffer to the cell material.
9. Transfer the entire content of the cryobag using a 50-mL syringe into a centrifuge tube.
10. Rinse the cryobag with 50-mL wash buffer and transfer it into the centrifuge tube as well.
11. Gently resuspend the cells with a 50-mL serological pipette.
12. Centrifuge the cells for 15 minutes at 300 x g at room temperature.
13. Carefully aspirate the supernatant after centrifugation, leaving a small amount of supernatant behind so that the cell pellet is not disturbed.
14. Resuspend the pellet with 50-mL cultivation medium, carefully until the suspension is homogenous.
15. Count cells and determine viability by preferred method.
16. Downstream experiments can be performed.

This protocol was developed based on internal procedures and equipment, but its suitability must be verified under your own conditions before use. From step 7 onwards, all steps are performed under sterile conditions in a biosafety cabinet.

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