



6 ML Yellow Top ACD Cryopreserved Whole Blood Processing

Purpose

This procedure describes cryo preservation processing of whole blood.

Safety Precautions

Exercise Universal Precautions. Latex or nitrile gloves and lab coat are required for the entire duration of the procedure. Perform this procedure in a safety cabinet (hood) using sterile technique.

Equipment and Supplies

Biological Safety Hood	72-grid test tube racks (white)
15 mL centrifuge tubes (1/ID)	Blue wire racks
1.0 mL Nunc cryo vials (6/ID)	KimWipes
1.0 mL pre-drilled blank (1/day)	Alcohol pads
DMSO in 50 mL centrifuge tube	Cryopreservation delete sheet
Eppendorf pipettor and pipette tips	White cryovial cap inserts
Transfer pipettes (1/ID)	60-grid workstation rack
Rocker	

Procedure

1. **Label** – Apply a temporary label or write the subject ID on one 15 mL centrifuge tube per ID. Apply barcoded labels on six 1.0 mL Nunc cryo vials for each subject ID. Place a white cap in half of the vials in order to separate the vials into two separate storage boxes.
2. **Mix** – Place the ACD whole blood on a rocker for at least five minutes.
3. **Prepare** – Pour a working volume of DMSO into a clean 50 mL tube clearly labeled with the substance name and date. ***Never aliquot any substance into an unmarked container. Do not allow the rim of the DMSO bottle to come in contact with the 50 mL tube.***
4. **Align** – Remove the ACD tubes from the rockers and arrange the tubes in numerical order by subject ID matched with a 15mL tube. Place each original

tube behind the 15 mL centrifuge tube that corresponds with that subject ID. Transfer the racks of ACD tubes and 15 mL centrifuge tubes to the hood.

5. **Decant** – Open the top of the ACD tube using a KimWipe. Pour the contents of the ACD tube into the labeled 15 mL centrifuge tube for that subject ID. Use an Eppendorf pipettor and 1-mL pipette tips to add DMSO in a 1:10 ratio to amount of whole blood in tube. (For example, if there is 6.5 mL of whole blood in the centrifuge tube, add 650 μ L of DMSO). Invert the tube at least ten times to mix the blood and DMSO thoroughly. Transfer the tubes to a designated rack immediately after adding the DMSO. Discard the rubber stoppers, empty tubes, and KimWipes into a biohazard waste container
6. **Aliquot** – Aliquot the ACD mixture into the labeled 1.0 mL cryovials, using an individually wrapped transfer pipette for each subject ID.
Note: Place cryovial caps down on clean Kim wipes, never on the cryovial rack or on the bare counter.
7. **Create Blank** – Create a blank for the CryoMed Cryopreservation Freezer by aliquotting approximately 1.0 mL of remainder blood into a pre-drilled 1.0 mL cryovial. These vials are located in a labeled box to the right of the hood.
8. **Freeze** – Mix the whole blood with DMSO for at least 10 minutes before specimens are cryopreserved. Freeze Specimens within one hour of the addition of DMSO. If specimen allocation cannot be completed in this timeframe, aliquot and freeze specimens in two or more batches. Freeze all cryovials in the CryoMed Cryopreservation Freezer.
9. **Store** – After the samples have reached -80°C , transfer the cryo vials to their final storage boxes in the -80 freezer. Incomplete boxes should be placed on Shelf 1. Complete boxes should be placed on Shelf 2 to await scanning. Scanned boxes should be placed on shelf 4 for transfer to repository.
10. **Delete** – If any cryovials were not filled, remove the barcoded labels and place them on the cryopreservation daily delete sheet. These aliquots will be removed from the database.