WHITE BLOOD CELL PREPARATION FOR CYSTINE DETERMINATION

Note: Refrigerate or freeze reagents upon arrival

Supplies provided by Biochemical Genetics/Cystine Lab:

ACD-Dextran solution	15 ml centrifuge tubes
0.9% NaCl and 3.6% NaCl	Empty Eppendorf tube with label
Distilled water	
0.1 ml 12% 5-sulfosalicylic acid (SSA) in Eppendorf tube	Request for Analysis

Additional supplies to be provided by your lab

Refrigerated centrifuge set at 5° C and capable of spinning at 450 x g	Dry ice/ethanol bath (styrofoam cup with walnut size piece of dry ice)
Vortex mixer	Warm water
Timer	Styrofoam container, strapping tape
Transfer pipets	Dry ice for shipping sample
Pipets for dispensing 0.8, 2.4 and 0.3 ml	Payment for shipping sample

We need to know the time of the patient's last medication and the time of the blood draw. (Optimum interval is 5-6 hours)

SHIPPING SAMPLES:

1. SHIP SAMPLES MONDAY TO WEDNESDAY ONLY.
2. Make sure the sample is labeled with the patient name and date.
3. Wrap the sample to protect it during shipping and place it in a styrofoam container with a minimum of 5 pounds dry ice.
4. Add the requisition form, including the time of last medication and blood draw**.**
5. Send Priority or Standard overnight (not First Overnight). Samples should be sent to the following address:

University of California, San Diego
Biochemical Genetics/Cystine Laboratory
CTF – Bldg B, Room 213
212 Dickinson Street
San Diego, CA 92103
(619)543-5260

6. Please do not return unused bottles or reagents.

BEFORE DRAWING THE BLOOD:

- ACD-Dextran should be at room temperature
- 9% NaCl, 3.6% NaCl and distilled water should be cold; keep on ice
- Determine the correct setting for your centrifuge (see next page). Set the temperature to 5° C
- Use a ballpoint pen to write the patient's name and date on the label provided

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Centrifuge settings for preparation of white cell pellet

r (inches)	rpm	r (inches)	rpm
3	2300	8	1400
4	2000	9	1300
5	1800	10	1250
6	1600	11	1200
7	1500	12	1150

r = inches from spin axis to center of tube, rpm = revolutions per minute

WBC ISOLATION PROCEDURE:

1. Draw 5 mls of heparinized blood (0.2 ml heparin, Na or Li) in syringe or a green top vacutainer.
2. **Immediately** transfer blood to the 15 ml centrifuge tube, add an equal volume of ACD-Dextran, cap and invert to mix. Place the blood-ACD mixture on ice for 30-45 minutes.

*If the blood cannot be processed immediately, hold at room temperature for **no longer than 60 minutes**. Standing markedly decreases the yield of WBCs.*

Keep everything cold. Do not stop during the procedure.

3. Transfer supernatant (it will appear cloudy and pink to amber) to a clean 15 ml centrifuge tube. It is more important to avoid taking red cells than to take all of the supernatant. The final value is based on protein, not volume.
4. Centrifuge at 450 xg for 10 minutes at 5° C.
5. Discard supernatant (the WBC pellet will look red).
6. Add 0.8 ml of 0.9% NaCl and 2.4 ml of distilled water. Vortex continuously at moderate speed for 90 seconds.
7. Add 0.8 ml of 3.6% NaCl. If there is a small red cell clot, remove it.
8. Spin for 3 minutes at 5° C. Discard the supernatant.
9. **Repeat steps 6 thru 8.**
10. Add 3.0 ml of 0.9% NaCl. Gently resuspend pellet by vortexing (pellet does not have to be completely resuspended). Spin for 3 minutes.
11. Discard supernatant without disturbing the white cell pellet.
12. Add 0.3 ml of the distilled water. Resuspend gently and transfer all of the cell suspension to a clean, labeled Eppendorf tube. If pellet is gummy and resists suspension, it may help to cut off the end of a clean blue tip for use in transferring. Transfer **everything** to the Eppendorf tube, even if it remains a glob.
13. Lyse the white cells by freezing in a dry ice/ethanol bath for 2 minutes and then in warm water for the **minimum** time necessary to thaw. **This is a critical moment**; The frozen cells and lysozomes will break open mixing their contents. Cysteine, glythathione, thiol groups on proteins, and the cystine we want to measure may react with one another. Proteins may hydrolyse. For this reason the thawing part of the freeze/thaw cycle should be as short as possible to keep the suspension cold and limit its reaction. Tube may be supported by a wire loop or strip of aluminum foil. Repeat two more times.
14. Transfer all (0.1 ml) of the 12% SSA solution to the Eppendorf containing the lysed cells and briefly vortex to mix.
15. Freeze sample. The sample can remain frozen up to two weeks. The final volume should be 0.4 ml (lysed WBC pellet + 0.3 ml of water + 0.1 ml of SSA).
16. Freeze left over reagents.

For a competency check of new personnel, please contact the lab at (619) 543-5260 for more information. You may also call this number for questions regarding samples, shipment, or kits.