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Title: Cell Thawing Procedure

PRINCIPLES OF THE PROCEDURE:

Frozen PBMC can now be classified according to the pattern of certain cell surface and intracellular markers. These markers can be demonstrated by the use of monoclonal antibodies which are "tagged" by fluorochromes. These labeled cells are acquired using a flow cytometer. When analyzing the data from the flow cytometer, a cell marker pattern emerges which can be useful in classification, prognosis, and treatment.

SPECIMEN REQUIREMENTS:

Frozen PBMC that have been thawed down from whole blood collected in Sodium Heparin tube. Keep at -150 degrees.

CRITERIA FOR SPECIMEN REJECTION:

1. Any unlabeled specimen will be rejected. All specimens must be labeled with identifier and the date.

2. Any specimen will be rejected that comes to the flow core that is already thawed.

3. Samples with a viability of <85% will only be reported at the specific request of the ordering persons. These reports will include a disclaimer that the viability was low and the results are suspect.

REAGENTS:

1. Phosphate Buffered Saline Solution PBS. These are located on the inside door of the clinical refrigerator. (See procedure in this manual.)

2. Immunophenotyping panels. The panels in use are kept in small amber glass bottles located on the second shelf of the cold room and labeled according to the Panel Look Up Table.

3. LIVE/DEAD® Fixable Yellow Dead Cell Stain Kit for Flow Cytometry (Invitrogen cat # L-34959). Resuspend powder in 50ul of DMSO. Aliquot 10ul into 5 eppendorf tubes and freeze at -20C. When ready to use add 90ul of PBS per aliquot for a 1/10 dilution.

4. Deoxyribonuclease I (Sigma cat # D5025). Dilute powder to 5,000 units/ml in PBS. Store at -20C for up to one year. Thaw when needed.

5. Complete Media RPMI 1640 10% FBS (See procedure in this manual).

6. 70% Alcohol

EQUIPMENT & INSTRUMENTATION

12 by 75 test tubes.
Pipettors and tips.
Vortex
Absorbent towels for blotting
Centrifuge
15ml tubes
Rack
Water bath
COULTER AcT 10

PROCEDURE:

1. Put complete media into the 37C water bath to warm.

2. When cells are ready to be thawed place freezing vials into a 37C water bath, remove vial right before its contents are completely thawed.

3. Wash outside of vial with 70% Alcohol.

4. Pipette the cell suspension from the vial into 10mls of complete media. Next, wash inside of vial with 1 ml of complete media, and then add to rest of cells.

5. Add 40ul of 5,000 units/ug of Deoxyribonuclease I to the 10mls of cells suspension.

6. Gently mix cell suspension by inversion after adding the Deoxyribonuclease I.

7. Incubate in 37C water bath for 30 minutes.

8. Wash with PBS and Centrifuge @ 1400 rpm (423 X g) for 5 min., aspirate supernatant carefully. Mix cell suspension well. Repeat this wash with PBS and repeat centrifugation.

9. Resuspend the cells into 2mls of PBS. Add blocking reagent if applicable to experiment.

10.

11. Obtain a cell count on the AcT-10.

12. Determine the amount of cells that will be needed from the AcT-10 count for the amount to equal one million cells per tube. (example: X ul(AcT10 count total) = $1x10^6$ cells X= number of ul needed per tube).

13. Add the specified amount to the test tubes calculated from number 11 above.

14. Add 5ul of the Live Dead Fixable Yellow Dye to each tube. Incubate on ice in dark for 15 minutes.

15. Follow the appropriate staining procedure that can be found in this manual.

PROCEDURAL NOTES:

INTERFERENCES: Protect stained cells from light to prevent quenching.
It is critical that no protein be in the tube, other than cells when the live dead viability probe is first added.

QUALITY CONTROL GUIDELINES

As a new lot of Reagent is prepared it should be tested with that day's healthy donor or similar sample in parallel with current lot to confirm that both give comparable results.

EXPECTED VALUES:

Samples with viability less that 85% should be reported out as unacceptable.

REPORTING RESULTS & CALCULATIONS

The results are calculated based on the % gated, % of total and the number of cells/μl.
See reporting of results.

REFERENCES: NA

VERSION HISTORY:

Version	Effective Date	Section	Description of Revisions/Justifications
			•
2010.02.19		SRP	
2010.04.20		SRP	Revision to step 9 of adding blocking reagent.

Name / Title	signature	Date
Author: name / title Alexis Conway RT		
Reviewer: name / title Mary Beth Dell Supervisor		
Approved: name / title Paul Wallace Director		