TITLE: Staining Procedure for PS1 specimens

PRINCIPLES OF THE PROCEDURE:

To obtain sufficient cells to sort 0.75 X 10^6 cells that are CD3+ and 0.75 X 10^5 cells that are CD33 positive. These will be used to check for chimerism.

Isolate sufficient cells for molecular pathology to check for chimerism, after an allogenaic transplant.

SPECIMEN REQUIREMENTS:

Whole Blood (heparinized)

REAGENTS/SUPPLIES:

Ice

2% Dextran (Sigma catalog # D4876)

Add 20g Dextran Sulfate (100,000MW) to 1 liter PBS. Mix well, filter and pH to 7.2.

Ammonium chloride lysing reagent (made up daily in the Clinical Lab)

Phosphate Buffered Saline (Invitrogen catalog # 21300-058)

Mouse IgG (Caltag catalog # 10400).

Antibody panel PS-1 (FITC-CD3, PC5-CD33, APC-CD45)

0.5% formaldehyde (Polysciences catalog # 04018)

EQUIPMENT & INSTRUMENTATION:

1 15 ml centrifuge tube

1 50 ml centrifuge tube

1 12 by 75 falcon tube

37 degree Incubator

Refrigerator Centrifuge

Vortexer

Refrigerator

Timer

Coulter Act10

PROCEDURE:

NOTE: Before use, warm Dextran to 20-37°c

1. Label one 50 ml centrifuge tube with patient identifier.
2. Perform WBC count using the Coulter AcT10, attach this print out to the requisition
3. Mix well heparinized vacutainer(s) containing patient blood.
4. Transfer mixed blood to 50 ml centrifuge tube, (use 4ml). Note the volume on the requisition.
5. Add [illegible] of 2% Dextran to this tube (1 ml) [illegible]

6. Incubate at 37^{o} C for 15 minutes (older specimens may take longer, do not incubate longer than 30 minutes).
7. Draw off the WBC suspension (supernatant) and place into a labeled 15 ml centrifuge tube, record this volume on the requisition, and dispose of Red Blood Cell pellet.
8. Centrifuge the sample at 1500 x g (3200) for 3 minutes at 4 degrees.
9. Decant, blot, and vortex sample.
10. Add 15 ml of ammonium chloride lysing reagent, mix well.
11. Incubate at room temperature for 5 minutes.
12. Centrifuge the sample at 1500 x g (3200) for 3 minutes at 4 degrees.
13. Decant, blot, and vortex sample.
14. Add 15 ml of PBS, mix.
15. Centrifuge the sample at 1500 x g (3200) for 3 minutes at 4 degrees.
16. Decant, blot, and vortex sample.
17. Resuspend in 1 ml of PBS.
18. Perform a WBC count on this sample. Attach the print out to the requisition.
19. Add 67 ul (3mg/mL) normal mouse IgG.
20. Incubate on ice for 10 minutes.
21. Calculate the amount of PS1 mAB to use.

    Multiply the 2nd count as on the printout by 10 and then
    Divide that number by 2.
    I.e. count is 16.2Lx10^{3}/ul
    Multiply by 10=162
    Divide by 2 = 81 ul of mAB

22. Label 12 x 75 mm plastic falcon tube with panel number and patient name.
23. Add amount of PS1 MAb calculated in step 21.
24. Mix and add all of sample to this tube.
25. Incubate on ice for 20 minutes.
26. Mix, wash with PBS.
27. Centrifuge the sample at 1500 x g (3200) for 3 minutes at 4 degrees.
28. Decant, blot, and vortex sample.
29. Add 1 ml of **0.5%** formaldehyde.
30. Incubate at room temperature for 20 minutes. **This is a critical step. DO NOT OVER INCUBATE**
31. Mix, wash with PBS.
32. Centrifuge the sample at 1500 x g (3200) for 3 minutes at 4 degrees.
33. Decant, blot, and vortex sample.
34. Resuspend in 1 ml of PBS.
35. Refrigerate until collected on the Aria.

PROCEDURAL NOTES:

Samples are stable for 7 days if fixed as described above and stored covered at 4-8^{o}c.

QUALITY CONTROL GUIDELINES: NA
EXPECTED VALUES: NA
REPORTING RESULTS & CALCULATIONS: NA
REFERENCES: NA

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