



<i>FILE NAME</i>	FL-SRP-2040.01 Surface Staining of Human Cells-
<i>VERSION</i>	FL-SRP-2040.02
<i>Replaces Version</i>	FL-SRP-2040.01

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TITLE: Surface Staining of Human Cells

PRINCIPLES OF THE PROCEDURE:

White blood cells, originally classified on the basis of morphology, can now be classified according to the pattern of certain cell surface 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 surface marker pattern emerges which can be useful in the classification, prognosis, and treatment of various diseases.

SPECIMEN REQUIREMENTS:

1. Bone marrow or whole blood must be collected in sodium heparin, sodium citrate, or EDTA tubes and kept at room temperature. Specimens must be processed within 48 hours of the time of collection; EDTA specimens within 24 hours.
2. Cerebral spinal fluid, pleural fluid and bronchial washes must be collected in sterile containers and kept at room temperature. Specimens must be processed within 6 hours of the collection time.
3. Tissue/Lymph node specimens should be sterilely minced using a scalpel and cut into sections from 0.01 to 0.25 cubic cm in size. They should be placed into a screw top, leak proof container with RPMI 1640 or alternatively HANKS Balanced Salt Solution (HBSS). Both should be supplemented with Gentamicin sulfate, 50 μ g/ml, to retard bacterial growth. Lymph nodes should be processed within 12 hours of collection. Tissue samples should be processed within 6 hours of collection and should be kept on ice or at 4°C. Samples with a viability of less than 85% will not be reported.

4. All requisitions must indicate:
 - a. Patient name and ID number
 - b. Date and time of collection
 - c. Sample type

CRITERIA FOR SPECIMEN REJECTION:

1. Any unlabeled specimen will be rejected. All specimens must be labeled with two patient identifiers, the date drawn and the sample type.
2. The requisition slip must have the time drawn; if it does not, the specimen will not be processed. Rather, we will call the appropriate individual for a time of draw. Once this is accomplished, the specimen will be processed.
3. Lymph node samples which are more than 12 hours old will be rejected.
4. Tissue samples, cerebral spinal fluid, pleural fluid and bronchial washes which are more than 6 hours old will be rejected.
5. Samples with a viability of <85% will only be reported at the specific request of the ordering physician. These reports will include a disclaimer that the viability was low and the results are suspect.
6. For a complete list of rejection criteria see *Criteria for Unacceptable Specimens SOP*.

REAGENTS:

1. Ammonium Chloride Lysing Reagent.
2. Phosphate Buffered Saline Solution PBS and PBS plus-Heparin (Gibco Cat# 21300-058).
3. Heparin Sodium (APP Pharmaceuticals LLC/ McKesson Health System car# 226-5403), use 1ml (1000 USP units) per liter of Phosphate Buffered Saline Solution PBS
4. Methanol Free Formaldehyde 10%. EM grade (Polysciences cat#04018). See procedure in this manual for dilution details
5. Immunophenotyping panels. The panels in use are kept in small amber glass bottles and labeled according to the Panel Look Up Table.
6. Mouse IgG. (Invitrogen cat# CUST MPKG).
7. LIVE/DEAD® Fixable Green Dead Cell Stain Kit for Flow Cytometry (Invitrogen cat# L-23101). See procedure in this manual for dilution details.

EQUIPMENT & INSTRUMENTATION:

12 by 75 test tubes
Pipettors and tips
Centrifuge adaptors
Absorbent towels for blotting
Vortex
Centrifuge
BD Falcon Cell Strainer 70um (Cat # 352350 BD)
COULTER AcT Diff
Test tube racks
Timer

PROCEDURE:

1. Perform a WBC on each sample (see Coulter Act Diff procedure in this manual).
2. Pipette 3ml (see sample dilution chart) whole blood or bone marrow filtered* from each patient's sample into a 15ml centrifuge tube and q.s. to 15ml with phosphate buffered saline containing heparin (1,000 USP units/liter).

*Filter all bone marrow samples using a 75 micron mesh

3. Centrifuge @ 1400 rpm (423 X g) for 5 min. at 7°C. Aspirate supernatant carefully. Mix cell suspension well. Repeat this wash with PBS (without heparin) and repeat centrifugation. Aspirate supernatant carefully. Repeat PBS wash and repeat centrifugation. Aspirate to a final volume of 2ml.
4. Add 67 µl (3mg/ml) normal mouse IgG for each ml of the above cell suspensions and incubate 10 min. on ice.
5. Label 12 x 75 mm plastic tubes with two patient identifiers, date and panel number (see SOP on how to make labels). Put labeled tubes in racks according to test requested.
6. Dispense antibody cocktails, standards, and special mAbs into all appropriately labeled tubes. Place on ice.
7. To the LIVE/DEAD tube add 5µl of the 1:50 dilution working stock solution. This solution will be placed in the Clinical Lab Refrigerator with the 4-Color antibodies.
8. Pipette 100 µl of the appropriate cell suspension into the appropriate tubes (labeled in 3 above) containing the dispensed antibodies and controls.

Rack or vortex each tube after adding cells to mAb's. Incubate in the dark on ice for 20 min.

9. Rack or vortex all tubes, two at a time. Place into centrifuge adaptors leaving a space in between each tube. Add 3.5 ml of lysing reagent, and place parafilm securely over the top of each adaptor. Invert twice. Let stand for 5 min at room temperature.
10. Centrifuge @1400 rpm (423 X g) for 5 minutes at 7°C. Carefully remove parafilm. Decant and blot each tube three times on a towel. Rack or vortex tube(s) and return to adaptors.
11. Add 3.5 ml PBS to each tube and centrifuge as above. Decant and blot as above. Rack or vortex each tube. Place tube(s) back into original racks.
12. Add between 0.3 to 0.5 ml (one drop of auto pipettor) of 2% formaldehyde to all samples. Cover with parafilm, and label rack with date.
13. Store in refrigerator no longer than 5 days until ready to put on the Flow Cytometer.

PROCEDURAL NOTES:

INTERFERENCES: Protect stained cells from light to prevent quenching.

QUALITY CONTROL GUIDELINES:

Blood from a Healthy Donor should be stained daily (excluding weekends) to ensure all panels are working properly. As a new lot of reagent is prepared, it should be tested with that day's healthy donor or similar sample in parallel with the current lot to confirm that both give comparable results.

EXPECTED VALUES:

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

REPORTING RESULTS & CALCULATIONS:

The results are calculated based on the percent gated, percent of total and the number of cells/ μ l.

REFERENCES:

1. Stewart, C. C. and Stewart, S. J. Cell Preparation for the Identification of Leukocytes. In: *Methods of Cell Biology*, Vol. 64, p. 218-70. New York: Academic Press, 2001.
2. Stewart, C. C. and Stewart, S. J. Multiparameter Data Acquisition and Analysis of Leukocytes by Flow Cytometry, Vol. 64, p. 289-312. New York: Academic Press, 2001.

VERSION HISTORY:

Version	Effective Date	Section	Description of Revisions/Justifications
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07.08.01	07.08.01	SRP	
FL-SRP-2040.01	3-5-10	SRP	Changed sample volume to 100ul. Plus corrections.
FL-SRP-2040.02	6-5-11	SRP	Updated

Name / Title	Signature	Date
Author: Title:		
Reviewer: Mary Dell Title: Clinical Lab Supervisor		
Approved: Paul Wallace Ph.D Title: Director		