Erythrocyte Lysis Buffer for Flow cytometry
(Investigation Use Only, IUO)
Ref: APELBFCK1

Introduction:
Prior to using cell suspensions for flow cytometric analysis and/or for in vitro functional assays, it is recommended to remove red blood cells (RBCs). The AP-RAD 10x RBC Lysis Buffer, containing ammonium chloride, is formulated to lyse Red Blood Cells from human and murine samples while leaving the lymphocytes, leukemic cells, hematopoietic precursors and granulocytes intact. This osmotic lysis method does not harm the white cells or alter membrane asymmetry. Due to the osmotic method of lysis, the 10X stock must be diluted to 1X with water. Using a buffer such as PBS alters the osmolarity of the lysis buffer and it will no longer be effective.

Kit content:
- Erythrocyte lysis Buffer 10x , 50 ml
- paraformaldehyde solution 1% , 50 ml

Storage and Stability:
Store the product at 2°c to 8°c until the expiration date printed on the label.

Precautions:
This reagent is designed for research purposes only. It is not intended for human or diagnostic use.
Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals.

Specimen collection:
Collect blood by standard venipuncture into an appropriate anticoagulant. Bone marrow aspirate mainly collected by hematologist – oncologist. Use EDTA or heparin (10 iu/ml) as anticoagulant. Do not use citrate, this produces an acidic environment which will quench your fluorescein labeled antibody!
Collect blood with a large gauge needle into a syringe. Do not subject the sample to large stresses by pulling too hard on the syringe. If you do this you will certainly damage erythrocytes and probably also damage leukocytes.
You may collect the sample directly into anticoagulant in the syringe. If you collect in a syringe without anticoagulant, carefully introduce the blood into a tube containing anticoagulant. Do not allow the sample to froth in either case.
However you collect the blood, ensure that is it completely mixed with anticoagulant .mix by inversion at least 10 times.
If you are interested in "resting" levels of antigens, immediately put the tube of blood into ice. Keep cold throughout.

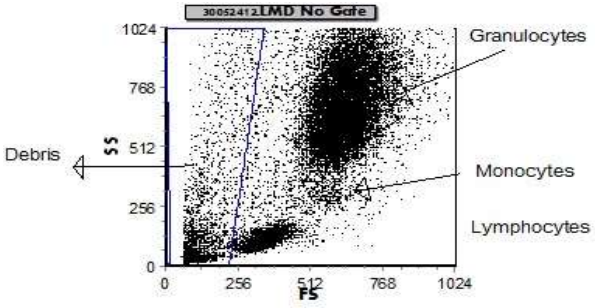
Procedure:
1. Mix 5 ml 10x buffer and 5 ml paraformaldehyde solution 1% and dilute the mixture to 50 ml using deionized water.
2. Warm the solution to room temperature.
3. Add the Fluorescent conjugated antibodies to stain directly to the sample.
4. Aliquot your sample of whole blood or Bone marrow aspirates into a tube. Mix well and incubate at room temperature for 30 minutes, protected from light.
5. Add 2 mL of room temperature 1X buffer and pulse vortex.
6. Incubate for 10 to 15 minutes, protected from light at RT (or 37°C to speed up the process). [1]
7. You can read directly by Flow cytometer or continue with Centrifuge at 500 x g for 5 minutes at room temperature and pour out the supernatant.
8. Resuspend the pellet with 1X PBS BSA (PBS containing 2% Fetal Bovine Serum and 0.02% Sodium Azide).
9. Centrifuge cells and resuspend to proceed with further analysis.

Acquisition buffer: Filtered PBS + 0.2 BSA + 2mM EDTA (NaN3 free) or BD FACS flow solution.

Quality control:
- To control erythrocyte lysis solution, expose the normal peripheral blood to lysis solution according to mentioned protocol (step 4-9) without any antibody and then analyze it by flow cytometry machine in SSC vs. FSC mode
- In ideal condition three population of cells including lymphocytes, monocytes and granulocytes are isolated as following table-1 and cytogram-1.

Table 1. Normal leukocytes according to their size and granularity

PB/BM/BF (Cells)	**FSC (size)**	**SSC (granularity & nuclear lobulation)**
Lymphocyte	Low	Low
Monocyte	High	Intermediate
Granulocyte	Intermediate	High



Cytogram 1. Different normal leukocyte Population

References:

1. http://flowcyt.salk.edu/protocols/wholeblood.html
2. Modified from the Handbook of Flow Cytometry Methods, J. Paul Robinson, editor, Wiley-Liss Inc

Table of Symbols	
LOT Number	LOT
REF Number	REF
Mfg. Date	
Exp. Date	
Read Pack Insert	
In Vitro Diagnostic	IVD
Investigation Use Only	IUO
Stored at	
Manufactured	

TECHNICAL SUPPORT AND ORDERING INFORMATION:
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[1] Stop incubation as soon as red blood cells are lysed. Prolonged incubation may alter cell viability.

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