

 IMMUNE TOLERANCE INSTITUTE	Document Title Whole Blood Washing Method			Document ID ITI-00046	Revision 1.0
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1.0 PURPOSE

1.1 This procedure describes the process for washing whole blood samples before staining to remove soluble components that may interfere with antibody binding to target antigens on leukocytes.

2.0 SCOPE

2.1 This SOP applies to all ITI core labs in all locations.

3.0 RESPONSIBILITIES

3.1 Cellular Analysis

3.1.1 ITI flow core lab operators follow this SOP to prepare washed whole blood samples for staining

3.2 QS

3.2.1 Reviewing and approving this document.

3.2.2 Notifying initiating department that biennial review is due for an SOP and their associated Form.

4.0 RELATED DOCUMENTS

4.1 ITI-00056, Trial specific assays

4.2 ITI-00045, Versalyse Whole Blood Staining Method

4.3 ITI-00067, FORM-Individual Sample Process Sheet

4.4 Sample Requisition Form (From ITN Sites)

5.0 DEFINITIONS

BEC	Beckman Coulter, Inc
HC	Healthy control
FCS	Fetal calf serum
huAB	Human AB serum
ITI	Immune Tolerance Institute
ITN	Immune Tolerance Network
PBS	Phosphate buffered saline
RT	Room temperature
WB	Whole blood

6.0 SAFETY

6.1 Follow all ITI safety policies and procedures. The processing of human whole blood is considered a potential biohazard. Treat all human blood as infectious material and process with proper protection. All waste should be disposed of in biohazard waste containers. Lab coat, goggles and gloves should be worn at all times

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7.0 EQUIPMENTS AND MATERIALS

- 7.1 Single channel pipettes (Rainin, RL20, RL100, RL200, RL1000 or equivalents)
- 7.2 Disposable pipette tips (Rainin disposable tips or equivalents)
- 7.3 Biosafety Hood
- 7.4 Centrifuge capable of 500 x g equipped with swinging bucket rotors, adapters for 12x75 tubes and biohazardous containment covers (Allegra-X15R Centrifuge or equivalent)
- 7.5 Vacuum with aspiration line and trap containing bleach in the waste receptacles
- 7.6 Eppendorf Repeater Plus (VWR, Cat#21516-002) or equivalent
- 7.7 Combitips 2.5mL, 25mL, and 50 mL (VWR, Cat# 22-26-740-0; 22-49-613-1; 22-26-770-1) or equivalent
- 7.8 Sterile serological pipettes 10mL, 25ml (VWR, Cat# 82050-482; 82051-182) or equivalent
- 7.9 Drummond Portable Pipette aid (VWR, Cat# 53498-103) or equivalent
- 7.10 Sterile 15mL or 50mL centrifuge tube (VWR, Cat#21008-103) or equivalent
- 7.11 Gloves (e.g. small, medium and large)
- 7.12 PBS Ca++ and Mg++ free (Invitrogen Cat #14190-136) or equivalent. Stored at RT
- 7.13 7AAD (Beckman Coulter PN A07704). Stored at 4°C
- 7.14 Heat inactivated fetal calf serum (FCS, Any vendor. Large stock stored at -80°C freezer and small aliquots at 4 °C refrigerator)
- 7.15 Heat inactivated Human AB serum (huAB, provided by ITN for ITN trial samples. Large volume stock at -80°C freezer and small aliquots at 4 °C refrigerator).
- 7.16 Whole blood (test samples, healthy controls, etc)
- 7.17 Trial specific data binders

8.0 PROCEDURE

- 8.1 For ITN samples huAB serum will be used. For other purpose FCS can be used.
- 8.2 Prepare 2%huAB serum in PBS
 - 8.2.1 Prepare enough 2%huAB/PBS for the amount of whole blood being washed (about 3xWB volume)
 - 8.2.2 To prepare 10 ml 2%huAB/PBS
 - 8.2.2.1 Label a 15 mL conical tube as 2%huAB/PBS
 - 8.2.2.2 Add 10 mL PBS to the tube
 - 8.2.2.3 Add 200µL of huAB serum (working stock stored at 4°C) and mix well
 - 8.2.2.4 2% huAB/PBS is only good at RT on the day it is made
- 8.3 Wash 1

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- 8.3.1 Dilute 1 part whole blood to 4 parts 2% huAB/PBS (For example, 300 μ L of blood plus 1.2mL 2%huAB/PBS) and mix well
- 8.3.2 Centrifuge the diluted blood at 300 x g for 10 minutes at RT
- 8.3.3 Carefully aspirate the supernatant. **Be careful not to suck out blood samples by leaving 2-3 mm buffer above blood line.**
- 8.4 Wash 2
 - 8.4.1 Add 2% huAB/PBS back to the volume in Step 8.2 (For example, 1.5 mL).
 - 8.4.2 Centrifuge the blood at 300 x g for 10 minutes at RT.
 - 8.4.3 Carefully aspirate.
- 8.5 Wash 3
 - 8.5.1 Add 2% huAB/PBS back to the volume in Step 8.2 (For example, 1.5 mL).
 - 8.5.2 Centrifuge the blood at 300 x g for 10 minutes at RT.
 - 8.5.3 Carefully aspirate.
- 8.6 Resuspend: restore blood to its original volume using 2% huAB/PBS (For example, 300 μ l).
- 8.7 The washed WB is ready for staining according to ITI-00045-CA-SOP-Versalyse Whole Blood Staining Method or other appropriate procedure.
- 8.8 Document any deviation in ITI-00067, Individual Sample Process Sheet and have lab supervisor review and sign the sheet with 24 hr of sample process.

9.0 RECORD RETENTION

- 9.1 ITI-00067, Individual Sample Process Sheet will be filed in Trial specific data binder

REVISION HISTORY				
Superseded Revisions		Document Change Number		Effective Date
NA				
Current Revision:	1.0	DCN:	10-026	
Section Number	Description of Changes		Justification of Changes	
All	New ITI document		Conversion of Beckman Coulter PRD-029-AA-Whole Blood Washing Method	