

# TBNK Flow Cytometry Assay Kit

**Catalog number:** 137-00020

User Manual

Last Revised: Jun 18, 2024

## Introduction

The RayBio® Human TBNK Flow Cytometry Kit is designed to determine the percentages of mature human lymphocyte subsets in peripheral whole blood (WB). The subsets include T lymphocytes (CD3<sup>+</sup>), Helper T lymphocytes (CD3<sup>+</sup>CD4<sup>+</sup>), Cytotoxic T lymphocytes (CD3<sup>+</sup>CD8<sup>+</sup>), B lymphocytes (CD19<sup>+</sup>) and Natural killer (NK) lymphocytes (CD3<sup>-</sup>CD16<sup>+</sup>&56<sup>+</sup>). Our kit contains individually packed fluorochrome-labeled antibodies, antibody diluent, and lysing solution to minimize the red blood cells. This kit is easy and convenient to use, and a perfect tool for immunophenotyping peripheral lymphocytes of human whole blood.

## Kit Contents

Components	Catalog #	100 tests
RayBright® Violet 450 Anti-human CD19	136-08044-100	2.5 µl/test, 250 µl
RayBright® Violet 500 Anti-human CD8	136-09025-100	
RayBright® Blue 488 Anti-human CD3	136-14012-100	
PE Anti-human CD4	136-18014-100	
PerCP Anti-human CD45	136-16069-100	
APC Anti-human CD16 & CD56	136-25000-100	
Antibody Diluent	137-10002-2ML	2 ml x 2 vials
RayBio® 10X Whole Blood Lysing Solution	137-10003-20ML	20 ml

## Storage / Stability

Store kit at 4°C, protected from light for up to three months for full functionality.

## Additional Materials Required

1. Flow cytometer with violet, blue and red lasers
2. 1.5 mL polypropylene microcentrifuge tubes or similar
3. Fluorescent counting beads
4. 12 × 75-mm tubes
5. De-ionized (DI) H<sub>2</sub>O
6. Centrifuge
7. Vortex mixer

## Assay Protocol

1. Dilute the RayBio® 10X Whole Blood Lysing Solution 10-fold with de-ionized (DI) H<sub>2</sub>O to prepare 1X lysing solution.
2. Prepare antibody cocktail in a 1.5 mL tube by adding 2.5 µl of the antibody reagents one by one and 35 µl of the Antibody Diluent in the kit per single test. The final volume of antibody cocktail per single test is 50 µl.

**Note:** Do not re-use pre-made cocktails. Always prepare one extra test to take in account for any reagent loss during the procedure.

3. Label each 12 × 75-mm tube and add 50 µL of well-mixed, anticoagulated whole blood from each sample to the bottom of the tube.

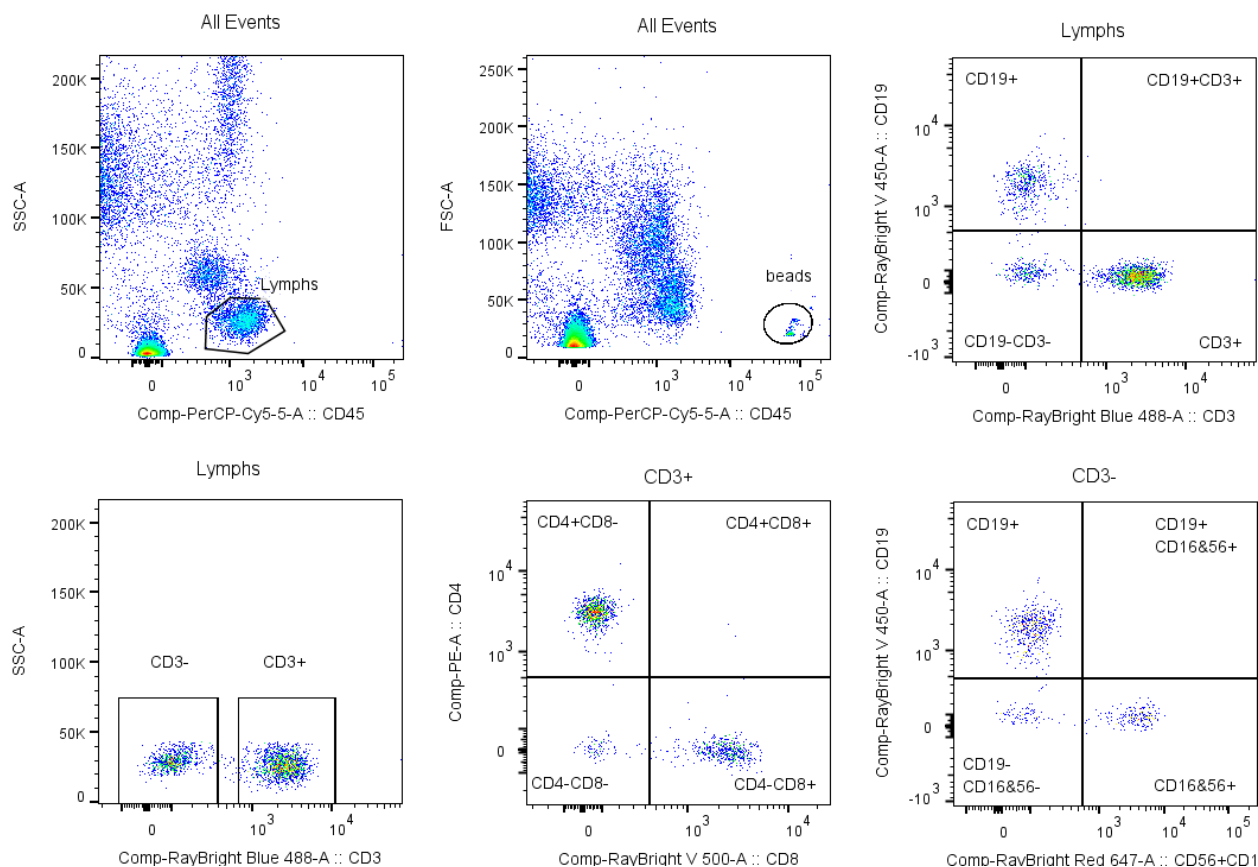
**Note:** Avoid pipetting the blood sample onto the side of the tube.

4. Pipette 50 µl of antibody cocktail from step 2 above into the bottom of each tube and vortex gently to mix.
5. Incubate for 20 minutes in the dark at room temperature (20°C–25°C).
6. Add 1 mL of 1X lysing solution from step 1 to each tube and vortex gently to mix.
7. Incubate for 20 minutes in the dark at room temperature (20°C–25°C).
8. Centrifuge the samples at 500 x g for 5 minutes and discard the supernatant without disturbing the pellet.
9. Add 0.5 mL of 1X lysing solution from step 1 to each tube. The sample is now ready to be analyzed on a flow cytometer.

**Note:** It's important to reduce aggregation before running samples on a flow cytometer.

## Results

A hematological normal adult whole blood sample stained with RayBio® Human TBNK Flow Cytometry Kit. Fluorescent counting beads are added for absolute number counts. See Figure 1 for representative images.



**Figure 1 Representative data collected using RayBio® Human TBNK Flow Cytometry Kit**