ReadiUse™ 6-Color Human TBNK Antibody Kit *Dry Reagent Format*

Catalog number: 90010, 90011
Unit size: 5 Tests, 25 Tests

Component	Storage	Amount (Cat No. 90010)	Amount (Cat No. 90011)
Component A: TBNK Dried Reagent	Room temperature (10-25 °C), Minimize light exposure	5 Tubes	25 Tubes
Component B: 10X Lyse/Fix Buffer	Room temperature (10-25 °C), Minimize light exposure	1 Bottle (0.3 mL)	5 Bottles (0.3 mL/bottle)

OVERVIEW

The ReadiUse™ 6-Color Human TBNK Antibody Kit for flow cytometry utilizes dried multicolor beads to delineate principal lymphocyte subsets - CD4+ T cells, CD8+ T cells, B cells, and NK cells – in whole blood specimens. Each TBNK dried reagent contains a cocktail of fluorescently-labeled monoclonal antibodies directed against human CD3, CD4, CD8, CD16, C19, CD45, and CD56. The kit is designed to be used in a "lyse/no-wash" format for quick and easy sample preparation, with less inconsistencies. Also included in this kit is a viability stain to identify dead cells and a precise number of fluorescent counting beads to determine absolute lymphocyte subset cell concentrations.

AT A GLANCE

Instrument
ReadiUse™ 6-Color Human TBNK Antibody Kit is designed to be used on a flow cytometer equipped with appropriate computer hardware and software. The flow cytometer must be equipped with:

1. 488 nm Blue Laser and 635 nm Red Laser
2. Capable of detecting light scatter (forward and side)
3. Four-color fluorescence channels when excited by the 488nm laser: FITC, PE, PerCP-Cy5.5, & PE-Cy7
4. Two-color fluorescence channels when excited by the 635nm laser: APC & APC-Cy7
5. Optional: 405nm Violet Laser and equipped with 455nm detection channels

Specifications

Laser	Emission	Conjugate	Suggested Channel (Example: Cytek Aurora)
Blue	520 nm	CD3 (SK7) - iFluor™ 488	B2 (518-529 nm)
Blue	575 nm	CD16 (3G8) - PE, CD56 (MY31) - PE	B4 (560-583 nm)
Blue	700 nm	CD45 (2D1) - PerCP-iFluor™ 680	B9 (688-707 nm)
Blue	780 nm	CD4 (SK3) - PE-iFluor™ 750	B13 (772-795 nm)
Red	665 nm	CD19 (SJ25C1) - APC	R1 (652-669 nm)
Red	780 nm	CD8 (SK1) - APC-iFluor™ 750	R7 (772-795 nm)
Violet	455 nm	NucPO-1	V3 (451-466 nm)

Precautions

1. The reagent contains sodium azide. Sodium azide is harmful if swallowed (R22). Wear suitable protective clothing. If swallowed, seek medical advice immediately. Contact with acids liberates toxic gas. Azides should be flushed with large amounts of water during disposal to avoid deposits in lead or copper plumbing
2. The 10X Lyse/Fix Buffer (Component B) contains formaldehyde. Formaldehyde is toxic and suspected to be a carcinogen. Avoid inhalation or contact.
3. All blood specimens are considered biohazards. Handle them as if they are capable of transmitting infection and dispose of them with proper precautions and in accordance with governmental regulations.

PREPARATION OF WORKING SOLUTION

1X Lysis/Fix Buffer
Prepare the 1X Lysis/Fix Buffer by adding 3.0 mL of deionized water to one bottle of the 10X Lyse/Fix Buffer (Component B). Mix and relabel the bottle as 1X Lysis/Fix Buffer.

SAMPLE EXPERIMENTAL PROTOCOL

Sample Preparation

1. Prepare blood cell samples, either fresh blood collected in an EDTA tube or isolated PBMCs at a concentration no greater than 10^6 cells/mL can be used.
2. Remove the desired number of reagent tubes from the pouch and reseal the pouch.
3. Thoroughly mix the sample and dispense exactly 50 µL of sample into the designated reagent tube. Note the accuracy of the sample dispense will affect the accuracy of the absolute cell concentration determined.
4. Gently vortex each tube for 30 seconds to ensure complete solubilization of the dried reagent.
5. Incubate for 20 minutes at room temperature. Protect the tube from direct light.
6. Add 450 µL of 1X Lysis/Fix Buffer to each tube and vortex for 10 seconds. Return tubes to the dark for at least 15 minutes.
7. Vortex sample tube thoroughly at low speeds and load onto cytometer for analysis. The ReadiUse™ 6-Color Human TBNK Antibody Kit is designed to be used in a Lyse/No-wash format. If the sample is washed before analysis, the ability to determine absolute cell concentrations will be lost.
8. **Flow Cytometer Acquisition:** start and operate flow cytometer according to manufacturer's instructions. Adjust the threshold to minimize debris and ensure populations of interest are included. Analyze the data using the appropriate cytometer-specific software.

EXAMPLE DATA ANALYSIS AND FIGURES

Data generated from blood or PBMCs can be displayed following the suggested scheme.

Adjust Threshold
Before acquisition, adjust the FSC channel threshold to collect the events from beads. The beads have low FSC. Some of the beads may be lost if your debris box is too big.

Generate the following dot plots:

1. **View 1** : Plot forward scatter versus side scatter to separate the cells and beads from the bulk of the debris.
2. **View 2** : Plot Anti-CD45 PerCP-iFluor™ 680 (675-715 nm) versus side scatter (SSC–Linear scale) for the cell population to separate cells from remaining debris.
3. **View 3:** Plot Anti-CD3 iFluor™ 488 fluorescence (515-545 nm) versus side scatter (SSC–Linear scale) for the total cell population to identify the CD3+ T cell and CD3- lymphocyte populations.
4. **View 4:** Plot Anti-CD8 APC-iFluor™ 750 (750-810 nm; 635 nm excitation) versus Anti-CD4 PE-iFluor™ 750 (750-810 nm; 488 nm excitation) for the events gated as **CD3+** to identify the CD4+ T cell and CD8+ T cell populations.
5. **View 5:** Plot Anti-CD19-APC (655-685 nm) versus Anti-CD16/56-PE (562-587 nm) for the **CD3-** lymphocyte population to identify the B cell and NK cell populations.
6. **Viability:** Plot NucPO-1 (425-475 nm) versus side scatter for any cell population to determine the viable cells in that population.

The absolute count for each cell type can be calculated using the following equation:

1. Absolute cell concentration (cells/µL) = [Gated cell count X Total number of beads in tube]/[Number of gated beads X Sample volume aliquoted into tube]
2. **Example:** If the bead count per reagent tube is 50,000, the volume of blood tested is 50 µL, the number of gated beads is 3,000, and the number of gated CD4+ T-cells is 1,500 then the absolute CD4+ T-cell count is 500 cell/µL.
    a. **Absolute CD4 Cell Count =** (1500 x 50000)/(3000 x 50) = 500 cells/µL

Cell Subsets

Plots	Population of Interest	Populations of Interest
FSC vs. SSC	All events	All events
NUcPO-1 vs. SSC	NUcPO-1 -	Live Cells
Anti-CD45 PerCP-iFluor™ 680 vs SSC	CD45+	Lymphocytes
CD3-iFluor™ 488 vs SSC	CD45+ CD3+	CD3+ CD45+ T Lymphocytes
CD3-iFluor™ 488 vs SSC	CD45+ CD3-	CD3- CD45+ Lymphocytes
CD4-PE-iFluor™ 750 vs CD8-APC-iFluor™ 750	CD45+ CD3+ CD4+ CD8-	T Helper cells
CD4-PE-iFluor™ 750 vs CD8-APC-iFluor™ 750	CD45+ CD3+ CD4- CD8+	T Cytotoxic cells
CD56 CD16-PE vs CD19-APC	CD45+ CD3- (CD56+CD16)+ CD19-	NK cells
CD56 CD16-PE vs CD19-APC	CD45+ CD3- (CD56+CD16)- CD19+	B Lymphocytes

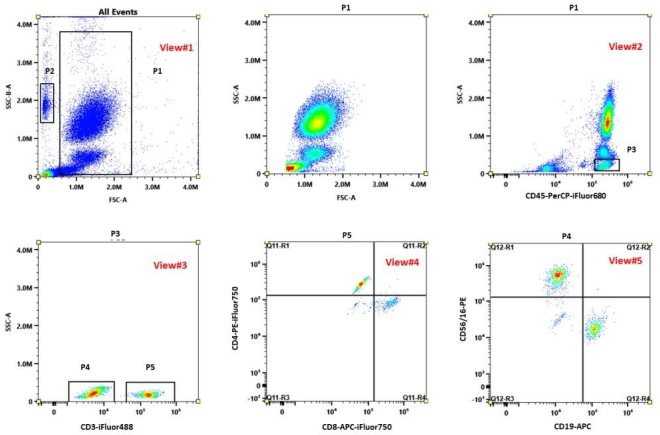

Figure 1. Human peripheral blood was stained with the ReadiUse™ Human TBNK 6 Color Antibody Kit *Dry Reagent*. Cells were stained at room temperature for 20 minutes, lysed with 1X Lysis/Fix buffer for 15 minutes, and then analyzed by flow cytometry. Live cells were gated on NucPO-1 viability stain. Live cells are gated on CD45+ (2D1)-PerCP-iFluor™ 680 for lymphocytes and then gated on CD3+ or CD3- (SK7) iFluor™ 488 cell populations. CD3+ cells are shown using CD4 (SK3) PE-iFluor™ 750 and CD8 (SK1) APC-iFluor™ 750 markers. CD3- cells are shown using CD19 (SJ25C1)-APC and CD56 (5.1H11) / CD16 (B73.1)-PE markers.

DISCLAIMER

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