

Operating Instruction For the Assay of TBNK lymphocyte Subsets

1. Operating steps

| Reagent | Dosage |
|-------------|----------------|
| CD45-FITC | 5 μ L/Test |
| CD3-PE-Cy5 | 5 μ L/Test |
| CD19-PE-Cy7 | 5 μ L/Test |
| CD16-PE | 5 μ L/Test |
| CD56-PE | 5 μ L/Test |

● No washing scheme

1. Add 5 μ L of each of CD45, CD3, CD19, CD16 and CD56 antibody reagents to the bottom of the flow tube.

(NOTE: The five antibodies can be mixed in a 1:1:1:1:1 ratio, then add 25 μ L of the mixture antibody to the bottom of the flow tube.)

2. Add 100 μ L mixed EDTA anticoagulation whole blood to the bottom of each test tube by reverse pipetting technique, avoiding blood touching the side wall of the test tube.
3. Vortex the tubes gently. Incubate for 20 minutes at room temperature (18~25°C), protected from light.
4. Add 1 mL of hemolysin to the test tube. Incubate for 15 minutes at room temperature (18~25°C).
5. Vortex the tubes gently before data acquisition on the machine.

● Washing scheme

1. Add 5 μ L of each of CD45, CD3, CD19, CD16 and CD56 antibody reagents to the bottom of the flow tube.

(NOTE: The five antibodies can be mixed in a 1:1:1:1:1 ratio, then add 25 μ L of the mixture antibody to the bottom of the flow tube.)

2. Add 100 μ L mixed EDTA anticoagulation whole blood to the bottom of each test tube by reverse pipetting technique, avoiding blood touching the side wall of the test tube.
3. Vortex the tubes gently. Incubate for 20 minutes at room temperature (18~25°C), protected from light.
4. Add 2 mL of hemolysin to the test tube. Incubate for 10 minutes at room temperature (18~25°C).
5. Centrifuge for 5 minutes at 300 \times g at room temperature (18~25°C).

6. Remove the supernatant by aspiration, with about 100 uL left in the test tube to avoid cell loss. Resuspend the cell pellet using 1 mL of PBS.
 7. Repeat steps 5 and 6.
 8. Vortex the tubes gently before data acquisition on the machine.
- (Note: Both washing scheme and unwashing scheme start red cell lysis from step 4. Different brands have different requirements. For the specific steps, please refer to the instructions of hemolysin used.)

2. Machine test conditions

Flow rate: medium speed;

Termination condition: Lym gate count 5000.

3. Flow reference diagram:

