Natural killer cell assay by Flow Cytometry General Protocol

(Shikhar Biotech)

1. Isolate PMBC from blood.
2. Re-suspend cells in 1 ml cold PBS.
3. Aliquot 100µl cell suspension in 5 tubes (0.5-1 million cells per tube).
4. Add primary antibody and other antibodies as shown below. (Please refer to product datasheet for recommended concentration), dilute in 1% BSA in PBST-if required.

   Set up appropriate compensation controls and experimental tubes as shown:

   Compensation tube 1: unstained cells
   Compensation tube 2: Add 1:200 diluted Anti-CD8^{AF488}
   Compensation tube 3: Add 1:200 diluted Anti-CD56^{PE} (BioLegend Cat# 362524)
   Compensation tube 4: Add 1:200 diluted Anti-CD3^{APC} (BioLegend Cat# 300312)
   Experimental Tube 5: Add 1:100 diluted Anti-CD56^{PE}, 1:200 diluted Anti-CD3^{APC} and 1:50 diluted goat Anti- NKG2D / KLRK1 (EB06839).

   For compensation control

5. Incubate for 30 minutes on ice in dark.
6. Wash with PBST 2 x 5 minutes.
7. To tube 5, add anti-goat IgG^{AF488} (Jackson Cat# 111-545-144) secondary antibody (diluted 1:100) in 1% BSA in PBS and incubate for 30 minutes on ice in dark.
8. Wash tube 5 with PBST 2 x 5 minutes.
9. Re-suspend cells in 200 µl PBS and analyze on CytoFlex flow cytometer.
10. Analyze data using CytExpert software.