SOP for Flow Cytometry

– Evaluation of Purified Antibody

Updated on 03.2025

1. **Experimental Instruments:** Analytical flow cytometer
2. **Experimental Materials and Reagents:**

   Various pipettes and tips, 96-well cell culture plates, light-shielding aluminum foil, NBS, primary antibody, fluorescent secondary antibody, 1×PBS, 0.2% BSA in 1× PBS (freshly prepared)
3. **Experimental Procedures:** The following procedures were prepared based on 30 samples. For different sample numbers, please adjust the volume of the buffers accordingly.

   3.1 Cell Preparation: Prepare approximately 2×10^5 cells per sample. Collect cells into centrifuge tubes, centrifuge at 300×g for 5 minutes, discard the culture medium, wash cells twice with 5 mL of 1×PBS, and discard the supernatant.

   3.2 Blocking: Resuspend cells in 4 mL 1×PBS, add 5% volume of NBS (400μL) to block cells, mix well, incubate at room temperature for 15 minutes, centrifuge at 300 ×g for 5 minutes, and discard the supernatant.

   3.3 Washing: Resuspend cells in 1.5 mL of freshly prepared 0.2% BSA, centrifuge at 300×g for 5 minutes, and discard the supernatant.

   3.4 Cell Plating: Resuspend cells in 1.5mL of 0.2% BSA, dispense cell suspension into

corresponding wells of a 96-well plate, 50 µl per well of cell suspension (approximately 2*10^5 cells).

3.5 Primary Antibody Reaction: Dilute the antibody to 2µg/ml and 20µg/ml, add to corresponding cell wells, 50µl per well, final volume 100µl, vortex to resuspend cells, incubate on ice for 30 minutes. (Antibody final concentrations are 1µg/ml and 10µg/ml, adjust concentrations according to cell expression levels). Note that these concentrations are recommended starting points. Users should adjust the concentrations based on their specific cell type and numbers.

3.6 Washing: Centrifuge the plate at 650×g for 3 minutes, flick off the supernatant, add 200µl of 0.2% BSA per well, centrifuge for 3 minutes, flick off the supernatant.

3.7 Secondary Antibody Reaction: Dilute the secondary antibody according to recommended concentrations, add to corresponding wells, 50µl per well, vortex to mix cells, incubate on ice in the dark for 30 minutes.

3.8 Washing: Centrifuge the plate at 650×g for 3 minutes, flick off the supernatant, add 200µl of 0.2% BSA per well, centrifuge for 3 minutes, flick off the supernatant, finally resuspend cells in 200µl of 0.2% BSA.

3.9 Detection: Select appropriate excitation light based on the fluorescent label of the antibody and perform machine testing.

Flow Result Analysis Demonstration

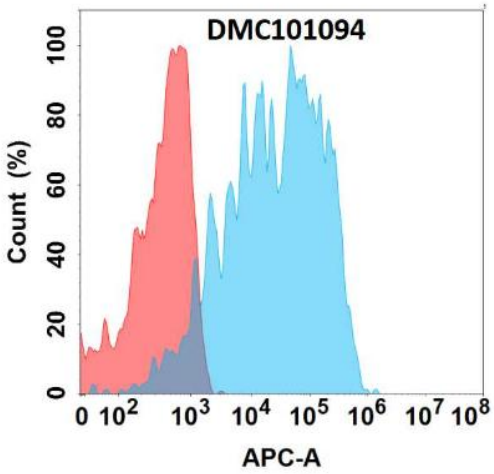

Figure 1. Flow cytometry analysis with 1μg/mL Anti-B7H7 (2D5) mAb on Expi293 cells transfected with human B7H7 (Blue histogram) or Expi293 transfected with irrelevant protein (Red histogram).

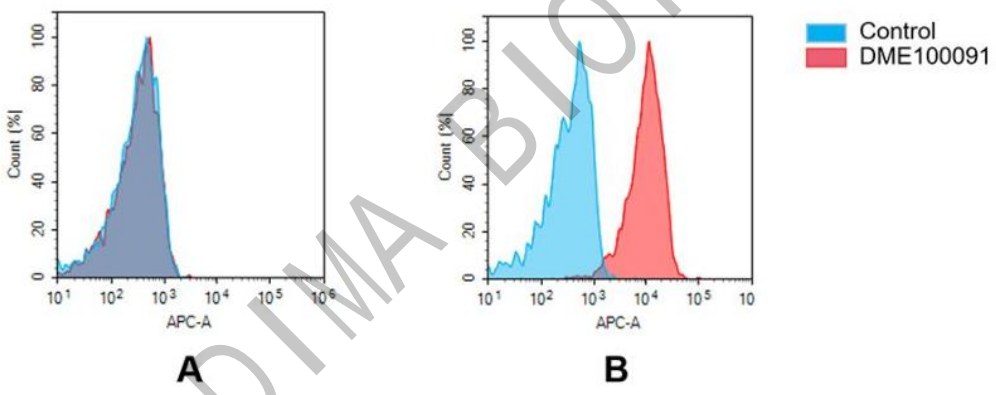

Figure 2 . Flow cytometry analysis of antigen binding of rabbit anti-human GPRC5D mAb (DME100091). (A) DME100091 does not bind to Jurkat cells that do not express GPRC5D. (B) A clear peak shift of DME100091 was seen compared to the control when incubated with GPRC5D-expressing MM.1S cells, indicating strong binding of DME100091 to GPRC5D. Antibodies were incubated at 5 μg/mL.