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FLOW CYTOMETRY PROTOCOL

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

PREPARATION

1. Harvest and wash the cells and determine the total cell number.
2. Resuspend the cells to approximately 1x106 cells/mL in ice cold PBS. Add the appropriate volume of paraformalde hyde to obtain a final concentration of 4%.
3. Fix for 10 minutes in a 37°C water bath.
4. Wash cells twice with PBS containing 0.5%BSA.
   Note: For extracellular staining with antibodies that do not require permeabilization, proceed to step 8; for intra cellular staining, proceed to step 5.

PERMEABILIZATION

5. Add ice-cold 90% methanol (approximately 1mL per 1x106 cells) and vortex.
6. Permeabilize for a minimum of 10 minutes on ice.

IMMUNOSTAINING AND ANALYSIS

7. Wash cells twice with PBS containing 0.5%BSA.
8. Resuspend 1x106 cells in 100uL PBS containing 0.5%BSA.
9. Add the primary antibody and incubate for 1 hour at RT.
10. Wash cells twice with PBS containing 0.5%BSA.
    Note: If using a fluorescent primary conjugated antibody, skip to step 14.
11. Resuspend cells in fluorochrome conjugated secondary antibody diluted in PBS containing 0.5%BSA.
12. Incubate for 30 minutes at RT.
13. Wash cells twice with PBS containing 0.5%BSA.
14. Resuspend cells in 0.5mL PBS and analyze on flow cytometer.