

MHC Staining Protocol

Procedure for Whole blood

1. Collect the blood into a tube containing anti-coagulant.
2. Lyse red blood cell using commercial red blood cell lysis reagent.
3. After red blood cell lysis, resuspend cells in FACS buffer (4% FBS in PBS) to a concentration of 1×10^7 cells/mL.
4. Add Dasatinib to the cells to a final concentration of 50nM and incubate 30 minutes.
5. Aliquot the cells into 100 μ L for each MHC sample. Add 1 μ g MHC tetramer and incubate for 30 minutes avoiding light.
6. If necessary, add any additional antibody (e.g anti-CD8) for cell sorting. Incubate for 30 minutes, avoiding light.
7. Centrifuge and remove the supernatant. Wash cells with FACS buffer.
8. Centrifuge and resuspend the cell pellet in 300 μ L FACS buffer and analyze by flow cytometry.

Procedure for Cell Preparations and Cell Suspensions

1. Prepare peripheral blood mononuclear cells (PBMC) or prepare a single-cell suspension from mouse spleen.
2. Centrifuge and resuspend the cell in FACS buffer (4% FBS in PBS). Adjust the cell concentration to 1×10^7 cells/mL in FACS buffer.
3. Add 50nM final concentration of Dasatinib into the cells and incubate for 30 minutes.
4. Aliquot the cells into 100 μ L for each MHC sample. Add 1 μ g MHC tetramer and incubate for 30 minutes, avoiding light.
5. If necessary, add any additional antibody (e.g anti-CD8) for cell sorting. Incubate for 30 minutes avoiding light.
6. Centrifuge and remove the supernatant. Wash the cell with FACS buffer.
7. Centrifuge and resuspend the cell pellet in 300 μ L FACS buffer and analyze by flow cytometry.

Additional Step: Peptide Pulsing

1. Prepare PBMC or mouse spleen cell under sterile conditions.
2. Using complete medium (RPMI 1640, 10% FBS, 500 IU/mL IL-2, 50 μ M β -Me, 1% P/S) dilute the cells to 2×10^6 cells/mL.
3. Dissolve the lyophilized peptide in DMSO at 10mg/mL. Add freshly dissolved peptide to a final concentration 10 μ g/mL in the medium. Incubate cells at 37°C for 72 hours.
4. After 72 hours incubation, feeding fresh complete medium every other day, cells can be grown for up to 2 weeks. The cells can be harvested for MHC staining after 7 days incubation, based on the cell counts.