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# Protocol: FACS-based Analysis for Mouse Autoantibodies

## Application:

A method to detect cell surface and intracellular autoantibodies from mouse serum or plasma.

## Preparation of Thymocytes:

1. Harvest and process mouse thymus per usual protocol and count total cells.

2. Split total cells evenly and strain through 70 μm filter into 2x 15 mL conical tubes.

3. Spin at 4 °C, 1600 revolutions per minute (rpm) for 5 minutes (min) and aspirate.
   *Note:* Unless otherwise specified, all centrifugation steps performed on a benchtop centrifuge.

3. Resuspend tubes in 5 mL of fixative.
   - Tube 1 in 4% Paraformaldehyde (PFA): fixation only for extracellular staining.
   - Tube 2 in Methanol (MeOH): fixation and permeabilization for intracellular staining.

4. Incubate for 1 hour.
   - Tube 1 (4% PFA) fixed cells at room temperature (RT).
   - Tube 2 (MeOH) fixed cells in a small ice bucket at -20 °C.

5. Add 5 mL of 1x PBS to each tube and spin as above.

6. Aspirate and resuspend cells in 5 mL of FACS buffer (1x PBS + 0.1% BSA) (2 tubes).

7. Spin again, aspirate and resuspend cells at *10 million (M) cells/mL* in FACS buffer (2 tubes).

8. Store cells at 4 °C (or coldroom).

## FACS-Based Detection Assay:

1. Aliquot 100 µL (~1M cells) of fixed thymocytes per well in a round bottom 96 well plate.
   - Relevant controls to consider:
     *(1)* cells only
     *(2)* cells + secondary antibody (Ab) (no serum/plasma added)
     *(3)* highly reactive positive control

2. Pre-dilute mouse plasma 1:50 before use.

3. Add 10 µL of pre-diluted serum/plasma to aliquoted thymocytes (final dilution of 1:500).
   *Note:* This dilution was dose determined for plasma obtained from 4 weeks old *Snai2*/*3* cDKO mice[1]. Optimal concentrations for other experiments may require titration.

4. Incubate at RT for 30 min in the dark.

[1] Pioli PD, Chen X, Weis JJ, Weis JH. Fatal autoimmunity results from the conditional deletion of Snai2 and Snai3. Cellular immunology. 2015;295(1):1-18. doi: 10.1016/j.cellimm.2015.02.009. PubMed PMID: 25732600; PMCID: 4617768.

5. Add 100 µL of FACS buffer to each well, spin 10 °C, 2000 rpm for 5 min and decant supernatant.

6. Add 200 µL of FACS buffer, re-spin and decant.

7. Resuspend wells (cells) in 100 µL of appropriate secondary antibody diluted in FACS buffer.
    a. **anti-mouse IgM-PE diluted 1:1000 -** (Thermo Fisher Scientific, Clone: eB121-15F9, [stock] = 0.2 mg/mL, Cat #: 12-5890-83)
    b. **anti-mouse IgG-AF488 diluted 1:5000 -** (Invitrogen/Life Technologies, [stock] = 2 mg/mL, Cat #: A11017)

8. Incubate RT 30 min in the dark.

9. Repeat steps 5 and 6.

10. Add 150 µL of FACS buffer followed by 150 µL of 2% PFA per well.

11. Strain/transfer cells into FACS tubes and assay using flow cytometer.