Immunofluorescent Staining of Frozen Murine Kidney Sections

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[Abstract] Renal immune complex deposition and leukocyte infiltration are characteristic of lupus nephritis in human patients and lupus-prone mice. This protocol describes how to stain frozen sections of murine kidney to study these features using fluorescent microscopy. This protocol was developed or modified in Dr. Anne Davidson's lab at Feinstein Institute for Medical Research.

Materials and Reagents

A. Antibodies
   1. Rat anti-mouse IgG2a FITC (Southern Biotech, catalog number: 1155-02)
   2. Rat anti-mouse IgG3 FITC (Southern Biotech, catalog number: 1190-02)
   3. Rat anti-mouse IgD PE (Southern Biotech, catalog number: 1120-09)
   4. Rat anti-mouse F480 PE (Life Technologies, Invitrogen™, catalog number: MF48004)
   5. Rat anti-mouse B220 PE (BD Biosciences, Pharmingen™, catalog number: BD553090)
   6. Hamster anti-mouse CD11c PE (BD Biosciences, Pharmingen™, catalog number: BD553802)
   7. Rat anti-mouse CD4 PE (BD Biosciences, Pharmingen™, catalog number: BD553049)

   Note: The above antibodies have been tested by the author and may be substituted with the antibodies desired by users.

B. Other materials
   8. Murine kidney tissues
   9. Acetone (Sigma- Aldrich, catalog number: 650501-4L)
   10. Tek O.C.T. Compound (Sakura Finetek, catalog number: 4587)
   11. 3% Fetal bovine serum (FBS) (Sigma- Aldrich, catalog number: F2442-500ML) in PBS.
   12. 0.5% Mouse BD Fc Block (BD Biosciences, Pharmingen™, catalog number: 553141)
   13. DAPI nucleic acid stain (Life Technologies, Invitrogen™, catalog number: D1306)
   14. Glycergel mounting medium (Dako, catalog number: C0563)
   15. Block solution

Equipment

1. Glass staining jars (Cole-Parmer, catalog number: EW-48585-00)
2. ImmEdge Pen (Vector Laboratories, Inc, catalog number: #H-4000)

Procedure

1. Murine tissues need to be snap frozen in Tek O.C.T. compound and cut into 5 µM sections.
2. Remove slides from -80 °C and keep in the dark at room temperature (RT) until thawed.
3. In the fume hood, immerse slides in acetone for 5 min and acetone needs to be pre-chilled in -20 °C.
4. Immerse slides in PBS for 5 min.
5. Repeat step 4.
6. Dry slides at RT and circle tissues with an ImmEdge pen.
7. Apply ~100 µl blocking solution per tissue and make sure that the solution is within the circles and the entire tissue is covered.
8. Incubate at RT for 30 min.
9. Dry the slides.
   Note: Do not dry the slides excessively.
10. Dilute the desired antibody 1/50 in 3% FBS/PBS.
11. Apply 100 µl antibody dilution per tissue to the slides.
12. Incubate the slides for 1 h at RT. Keep in dark.
13. Wash slides in PBS three times (5 min each wash). Keep in dark.
14. Apply 100 µl DAPI solution (300 nM) per tissue to the slides.
15. Incubate the slides at RT for 1-5 min. Keep in dark.
16. Wash the slides in PBS three times (5 min each). Keep in dark.
17. Heat glycergel mounting medium to 65 °C in a water-bath until melted.
18. Drain out the PBS from the slides.
19. Apply the mounting solution to the top of the slides and cover tissue with cover glass, making sure no air bubbles are formed.
20. Slides are now ready for immediate use for fluorescent microscopy or can be stored at 4 °C in dark for future use.

Notes

This protocol has been successfully used for staining murine renal immune complexes, renal leukocyte infiltrates (CD4 T cells, B220 B cells, F480 macrophages, and CD11c dendritic cells), and murine splenic structures such as B cell follicles (IgD^+) germinal centers (Peanut $Agglutinin^+$).

Recipes

1. Block solution
   0.5% Mouse BD Fc Block in 3% FBS

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References

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