Immunohistology of mouse spleens

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Method Article

Keywords: Immunohistology, mouse, spleen

Posted Date: January 22nd, 2007

DOI: https://doi.org/10.1038/nprot.2007.40

Abstract

Introduction

This protocol was used in our _Nature Immunology_ paper.

Reagents

OCT compound embedding medium \(Sakura, Finetek, Torrance, CA) Liquid nitrogen Superfrost Plus slides \(Fisher Scientific, Pittsburgh, PA) Coverslips \(Fisher Scientific, Pittsburgh, PA) Acetone \(histological grade, Sigma) PBS + 5% fetal calf serum Anti-CD3-phycoerythrin \(Pharmingen) Anti-B220-FITC \(Pharmingen) Anti-peanut-agglutinin-biotin \(US biological) Streptavidin-cyanin 5.5 \(Pharmingen) PBS + 0.05% Tween 20 \(Sigma) Gelmount \(Biomeda, Foster City, CA)

Procedure

Harvest of mouse spleens and preparation of splenic sections **1** Harvest mouse spleens, embed them in OCT compound embedding medium and freeze in liquid nitrogen. **2** Cut 7 µm sections and mount them on Superfrost Plus slides. **3** Incubate slides in humidified chambers overnight at 4 ˚C and dry them, then fix them in acetone at 4 ˚C for 10 min and block them with PBS + 5% fetal calf serum at 25 ˚C for 1 h. **Antibody staining and microscopy** **4** Stain blocked sections overnight at 4 ˚C with a combination of anti-CD3-phycoerythrin, anti-B220-FITC, and peanut-agglutinin-biotin, all diluted in blocking buffer \(all the antibodies need to be titrated for optimal results; dilution 1:200 – 1:1000) \(1). **5** Wash the slides 3x with PBS + 0.05% Tween 20. Stain sections with streptavidin-cyanin 5.5 \(dilution 1:100) at 25 ˚C for 1 h. **6** Wash the slides 3x with PBS + 0.05% Tween 20, dry them and mount them with Gelmount. **7** Capture images at x10 magnification with a Zeiss Axioplan microscope.

Timing

2 d

References

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Acknowledgements

Supported by the National Institutes of Health \(ES04151 and ES06376 to M.K.), the American Cancer Society \(M.K.) and the Leukemia and Lymphoma Society of America \(T.E.; SCOR grant 7332-06).