

## Basic Immunology Practice - 2026.

### Direct immunohistochemistry

During today's practice, you will become familiar with immunohistochemistry by performing a direct immunohistochemical staining procedure. Since you will be working with toxic chemicals, please wear a lab coat and rubber gloves.

A mouse spleen section is located on the slide. The isolation of the mouse spleen, its frozen sectioning, and fixation in acetone have already been performed by colleagues. On the slide, the spleen section has been circled with a hydrophobic marker to prevent the spreading of the liquid applied during the immunoreaction.

1. The first step is **inhibition of endogenous peroxidase** with phenylhydrazine. Since the phenylhydrazine solution is toxic, handle it only while wearing rubber gloves. Place a pipette tip onto the pipette and carefully pipette 100 µl of solution dropwise onto the circled section. Carefully dispense the solution onto the section. Make sure that the pipette tip does not touch the section, as it may easily become damaged. Use a new pipette tip for each step. Discard the used pipette tips into the waste container. The incubation time is 10 minutes.
2. After the incubation time has elapsed, pour off the phenylhydrazine solution from the slide. Turn the slide to the side and allow the solution to flow into the Petri dish. Always wipe the liquid from the other side of the slide but be careful not to wipe off the section.
3. **Washing step**: place the slide into the PBS I container. Incubation time: 2 minutes.
4. Remove the slide from the PBS solution, then repeat the washing step in the PBS II container. Additional incubation time: 2 minutes.
5. The next step is **blocking**. In this step, non-specific protein binding sites are blocked with 5% BSA-PBS solution. Remove the slide from the PBS solution and carefully pipette 100 µl of BSA-PBS solution onto it. After 10 minutes of incubation, pour off the liquid from the section (no washing step follows this step).
6. The next step is **the staining of the T cells**. T cells are labeled with an HRP-conjugated anti-Thy-1 (T cell marker) monoclonal antibody. Pipette 100 µl of the antibody solution onto the section. Incubation time: 30 minutes.
7. **Wash** the section twice for 2 minutes **with PBS** (in fresh PBS), as described above (steps 3–4).
8. The final step is **the addition of the chromogen and the substrate**. Carefully remove residual PBS from the section. The chromogen (DAB: diaminobenzidine, TOXIC) is dissolved in 0.1 M (molar) sodium acetate buffer in the presence of hydrogen peroxide (substrate). In the presence of the substrate, the HRP conjugated to the antibody converts the chromogen into a colored end product within a few minutes.
9. **Examine** the histological sample **under a microscope**.