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Immunohistochemistry: Preparation and Staining of Paraffin Sections: Formalin and Zinc Fixation

This protocol is for use with BD Pharmingen™ reagents. Before you begin, determine the fixation method (formalin or zinc) and the method of heating slides (microwave or pressure cooker or autoclave) for antigen retrieval, if needed.

I. Tissue Preparation

1. Sacrifice the animal according to prescribed and approved euthanasia techniques.
2. Cut the tissues to be fixed and processed to no thicker than 3 mm.

II. Fixation and Processing of Tissue for Paraffin Sections

A. Fixation of Tissues in 10% Neutral Buffered Formalin

1. Let tissues fix in 10% formalin at room temperature for 8 hours (4 to 8 hours for small-rodent tissue) but no longer than 24 hours.
2. Rinse with running tap water for 1 hour.
3. Follow the processing schedule recommended in section C.

B. Fixation of Tissues in Zinc Fixative

Many antigenic epitopes are masked or even destroyed by 10% formalin fixation. In some cases, fixation in a milder fixative such as BD Pharmingen™ IHC zinc fixative (Cat. No. 550523) is helpful to preserve the antigenic epitopes.

Use the following recommended fixation times:

Dense tissues such as cardiac muscle, skin, fat	24 to 48 hours
Lung, spleen, thymus, GI tissues	6 to 8 hours

Optimization might be required based on species and the size of the tissue.

1. Place freshly dissected tissues into zinc fixative and fix for the recommended time.
2. Rinse with running tap water for 30 to 45 minutes.
3. Follow the processing schedule recommended in section C.

C. Processing Schedule

The processing, embedding, and sectioning of paraffin blocks requires specialized equipment and expertise and is usually performed by a histology or pathology laboratory. While hand processing can be performed according to the following protocol, the results might show marked variation in histology quality and antigenicity.

Station	Time (min)	Solution
1		Water
2	45	70% alcohol
3	45	80% alcohol
4	45	90% alcohol
5	45	100% alcohol
6	60	100% alcohol



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7	60	100% alcohol
8	60	Clearing reagent (xylene or substitute)
9	60	Clearing reagent (xylene or substitute)
10	60	Paraffin 1
11	60	Paraffin 2
12	60	Paraffin 3

III. Preparation of Paraffin Sections for Immunohistochemistry

A. Sectioning Protocol

1. Section paraffin blocks at the thickness you want (usually 4 to 5 μm) on a microtome and float on a 40°C water bath containing distilled water.
2. Transfer the sections onto a Superfrost Plus slide. Allow the slides to dry overnight and store them at room temperature until ready for use.

B. Deparaffinization and Rehydration of Tissue Slides

1. Place the slides in a 55°C oven for ten minutes to melt the paraffin.
2. Deparaffinize the slides in two changes of xylene or xylene substitute for 5 minutes each.
3. Transfer the slides to 100% alcohol, make two changes for 3 minutes each, and transfer once through 95% alcohol for 3 minutes.
4. Block endogenous peroxidase activity by incubating sections in 3% H_2O_2 solution in methanol for 10 minutes.
5. Rinse in PBS twice for 5 minutes each time.
6. If the antibody staining requires antigen retrieval to unmask the antigenic epitope, continue with section C. If antigen retrieval is not required, proceed to section D.

C. Pretreatment of Paraffin Sections with Retrievacen A* (pH 6.0)

Microwave oven method**

1. Make a working solution of BD Pharmingen™ Retrievacen A by mixing 18 mL of solution 1 and 82 mL of solution 2. Bring the final volume to 1 liter in distilled water.
2. Place slides in a plastic coplin jar filled with the Retrievacen A working solution and heat in a microwave oven to 203°F (95°C).
3. Mix the Retrievacen A working solution in the coplin jar with a disposable pipet and incubate the slides at 203°F (95°C) for 10 minutes.
4. Remove the coplin jar with the slides, cover the jar tightly, and allow the solution to *slowly* cool to room temperature for 20 minutes to enable the protein molecules to fold properly.
5. Rinse the slides in PBS three times for 5 minutes each time.



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*For methods using other antigen retrieval systems, see the instructions in technical data sheets.

**Heating using a microwave oven may require a license under US patent No. 5,244,787.

Pressure Cooker or Autoclave Method

1. Prepare a working solution of Retrievalgen A as described in step 1 of the microwave oven method.
2. Place the slides in a glass or metal coplin jar and heat in a pressure cooker or autoclave at 120 to 125°C, 17 to 25 psi, for 5 minutes.
3. When completed (at 0 psi), open the pressure cooker or autoclave and allow the slides to cool to room temperature for approximately 20 to 30 minutes.
4. Remove the slides from the coplin jar and wash them as described in step 5 of the microwave oven method.

D. Immunohistochemical Staining of Paraffin-embedded Tissues

See *Standard Immunohistochemical Staining Procedure* (Section III of Immunohistochemical Staining of Frozen Sections)
http://www.bdbiosciences.com/support/resources/protocols/frozen_tissue.jsp.

Begin at step 5 and proceed through coverslipping.

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