

Antigen Retrieval Techniques: For use with F4/80 Immunostaining of Paraffin-Embedded Sections

Protocol

IHC9

Antigen unmasking procedures are recommended to achieve optimum IHC staining for F4/80, as the F4/80 antigen can be masked by prolonged formalin-fixation and the paraffin-embedding process.

Notes:

- The following two procedures should be used alongside the Indirect Immunostaining of Paraffin-Embedded Tissues protocol
- Recommended fixative for the F4/80 antigen is Neutral Buffered Formalin
- Appropriate controls should always be carried out
- Recommended positive control tissue: Liver and lymph node

Option 1. Enzyme pre-treatment using proteinase K

This procedure is recommended for tissues fixed for 24 hours in Neutral Buffered Formalin (NBF).

A number of buffers may be used for this purpose; the precise choice is usually recommended on the product specific datasheet or determined experimentally. For your convenience Bio-Rad offers a range of pre-prepared Antigen Retrieval Buffer at different pH levels guaranteeing consistency of performance and reliability.

Alternatively buffers may be prepared using the recipes listed below. For further details contact Technical Support.

Reagents:

1. TE buffer (50 mM Tris base, 1 mM EDTA, pH 8.0)

- Tris Base, 6.10 g
- EDTA, 0.37 g
- Distilled water, 1000 ml
- Mix to dissolve. Adjust pH to 8.0 using concentrated HCl (10 M HCl)
- Store at room temperature

2. 20 x Proteinase K stock solution (400 µg/ml in TE buffer, pH 8.0)

- Proteinase K, 4 mg
- TE buffer, pH 8.0, (Reagent 1.) 10 ml
- Mix well
- Store in aliquots at -20°C

3. 1x Proteinase K working solution (20 µg/ml in TE buffer, pH 8.0)

- 20x Proteinase K stock solution, (Reagent 2.) 1 ml
- TE Buffer, pH 8.0, (Reagent 1.) 19 ml
- Mix well
- Discard working solution after use

Method:

1. Dewax paraffin sections and rehydrate using preferred procedure.
2. Cover sections completely with Proteinase K working solution and incubate for 3 minutes at room temperature. (N.B. As the specific activity of Proteinase K can vary between batches, it can be advantageous to determine the optimal incubation time for the current batch by comparing incubation times of 2, 3 and 4 minutes in a test run).
3. Rinse sections with Phosphate Buffered Saline (PBS).
4. Optional step; if required proceed with endogenous peroxidase de-activation. Bio-Rad offer peroxide blocking reagent (BUF017B).
5. Proceed with preferred staining protocol.

Option 2. Heat-mediated antigen retrieval using citrate buffer, pH 6.0

This procedure is recommended for tissues fixed for 7 days or more in Neutral Buffered Formalin (NBF)

Reagent:

Citrate buffer (10 mM citric acid, pH 6.0)

- Citric acid (anhydrous), 1.92 g
- Distilled water, 1000 ml
- Mix to dissolve
- Adjust pH to 6.0 with 1 M NaOH (be sure to mix well)
- Store this solution at room temperature for 3 months, or at 4°C for longer usage

Method:

1. Dewax paraffin sections and rehydrate using preferred protocol.
2. Pre-heat sodium citrate buffer in a staining vessel to 95-100°C.
3. Immerse slides in the citrate buffer and incubate for 10 minutes at 95-100°C. Check the citrate buffer level, add more if necessary, and then incubate for a further 10 minutes at 95-100°C.
4. Allow sections to cool for 20 minutes.
5. Rinse sections with PBS.
6. Optional step; if required proceed with endogenous peroxidase de-activation. Bio-Rad offer peroxide blocking reagent (BUF017B).
7. Proceed with preferred staining protocol.

Benefits of using this antigen retrieval procedure can be seen below. Image A. shows IHC staining with no pre-treatment and image B. shows IHC staining that can be achieved after pre-treatment, clearly resulting in superior staining.

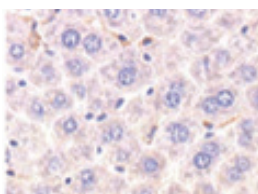


Image A. F4/80 antibody staining of liver tissue fixed in NBF for 28 days, no antigen retrieval.

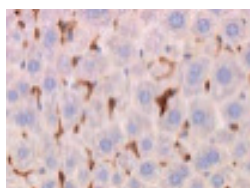


Image B. F4/80 antibody staining of liver tissue fixed in NBF for 28 days with citrate buffer & heat-mediated antigen retrieval.

Are you interested in learning more about which reagents to use in your immunohistochemistry experiments? Bio-Rad's immunohistochemistry application page enables you to quickly and easily find antibodies, kits and reagents tested in immunohistochemistry.

Find out more at: [bio-rad-antibodies.com/ihc-resources](https://www.bio-rad-antibodies.com/ihc-resources)



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