



# MMPC Immunohistochemistry

## Factor VIII

Version: 1

Modified from: IHC Methods and Materials VMTH - Anatomic Pathology, UC-Davis

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**Summary:** Factor VIII is a glycoprotein present in human plasma, human endothelial cells, megakaryocytes and platelets. Immunohistochemical staining for factor VIII related antigen could be used to determine if the benign and malignant neoplastic lesions are of endothelial origin. Furthermore, it can be utilized to determine vessel density in a tissue of interest.

### Reagents and Materials:

| Reagent/Material                      | Vendor          | Stock Number |
|---------------------------------------|-----------------|--------------|
| xylene                                |                 |              |
| ethanol                               |                 |              |
| Hydrogen peroxide                     |                 |              |
| Methanol                              |                 |              |
| Proteinase K                          | Dako            | S3020        |
| 0.1M Phosphate Buffered Saline pH 7.4 |                 |              |
| Normal horse serum                    |                 |              |
| Tween-20                              |                 |              |
| Factor VIII Ab                        | Dako            | A0082        |
| biotin-avidin based HRP               | Biocare Medical | GR608        |
| NovaRed for peroxidase                | Vector          | SK-4800      |
| Streptavidin-HRP HP604                | Biocare Medical | HP604        |
| Mayer's Hematoxylin                   | Dako            | S3309        |
| coverslip                             | Corning         | 2935-245     |

### Protocol:

**WARNING:**

*Formalin is, toxic, flammable and considered a carcinogen*

*Xylene, ethanol and methanol are all flammable and should be used in fume hood away from open flames or sparks*

*All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC when handling and disposing of infectious agents.*

1. Immunohistochemistry was performed on four-micron thick, formalin-fixed, paraffin-embedded tissue sections, mounted on charged slides, and air-dried overnight at 37° C.
2. Sections were deparaffinized through xylene to 100% reagent alcohol, and then treated with 0.3% hydrogen peroxide in 100% methanol for 30 minutes.
3. Sections were rehydrated to deionized water through 95% and 70% reagent alcohols. Antigen retrieval was performed on sections for IBA-1 with heat induced epitope retrieval in a Black & Decker Steamer using Target Retrieval Solution, pH 6 (Dako S1699) for 30 minutes at 95°C, followed by a 20 minute cool down.
4. Retrieval was performed on sections for Factor VIII with Proteinase K (Dako S3020) at room temperature for 10 minutes.
5. After antigen retrieval, slides were rinsed in deionized water and placed in 0.1M Phosphate Buffered Saline, pH 7.4 (PBS). The antibody diluent and blocking reagent were PBS-Tween 20 (0.02%) and 10% normal horse serum (NHS) in PBS-Tween 20, respectively.
6. Sections were blocked for 20 minutes with antibody diluent and primary antibodies were applied without rinsing and incubated for 1 hour.
  - a. All post-antigen retrieval incubations are in a humidity chamber at room temperature.
7. After primary incubation, samples are rinsed twice for three minutes with PBS-Tween 20 between each subsequent reagent application.
8. A single step, polymer based HRP (BioCare Medical, RC542H) was applied for 30 minutes to label rabbit anti-IBA-1. A dual step, biotin-avidin based HRP (Biocare Medical, 4+ Detection System GR608) was applied for 10 minutes to link rabbit anti-Factor VIII.
9. Streptavidin-HRP (Biocare Medical HP604) was applied for 10 minutes to label the biotin link.
10. All labels were visualized with NovaRed for peroxidase (Vector SK-4800), per manufacturer's instructions.
11. Sections are counterstained in Mayer's Hematoxylin, air dried and coverslipped.

## Reagent Preparation:

### Reagent 1: PBS-Tween 20

Reagents and Materials:

0.1M Phosphate buffered saline (PBS), pH 7.4

Tween 20

Procedure:

For 100mL combine 100mL PBS and 20uL Tween 20

### Reagent 2: Antibody diluent/blocking solution

Reagents and Materials:

PBS-Tween 20

Normal Horse Serum (NHS)

Procedure:

For 100mL combine 90mL PBS-Tween 20 and 10mL NHS.