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UC Davis - Immunohistochemistry Factor VIII V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.56qg9dw

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ABSTRACT

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

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

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=256&docType=Protocol

MATERIALS

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

MATERIALS TEXT

Reagent Preparation:

Reagent1: 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.

Note:

Biocare Medical, RRID: SCR-013549

Vector Laboratories, RRID:SCR_000821

Dako RRID:SCR_013530

Factor VIII Ab # A0082, Cite this, **(Agilent Cat# A0082, RRID:AB_2315602)**

SAFETY WARNINGS

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 37º 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 95ºC, 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.