Immunohistochemistry protocol for detection of fetuin-A via APAAP-Kit in older human autopsy tissue

PLOS One

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dx.doi.org/10.17504/protocols.io.sydefs6

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ABSTRACT

This staining was employed to detect Fetuin-A in paraffin sections (1 µm thickness) of formalin-fixed human brain tissue. For fetuin-A staining, we used a monoclonal IgG2a mouse-anti-human antibody (clone MAHS-1, dilution 0.1-0.5 µg/mL), raised against purified human fetuin-A in our laboratories. Antibodies were diluted in a 1% dilution of Bovine serum albumin (BSA) in phosphate-buffered saline (PBS) and were immediately applied to the re-hydrated sections. Bound antibody was detected using Dako REAL™ Detection System, which employs APAAP immunochemistry and fast red chromogenic substrate (Dako K5000, Glostrup, Denmark) following the manufacturers protocol. Counterstaining was employed with Mayer´s hematoxylin solution (Roth, T160.1, one minute). The slides were then washed in demineralized water and dehydrated in graded alcohol (concentrations from 70% to 100%). After placing in xylene the sections were mounted (Roth, T160.1) and covered using coverslips.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0206597

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Heinen MC, Babler A, Weis J, Elsas J, Nolte K, Kipp M, Jahnen-Dechent W, Häusler M (2018) Fetuin-A protein distribution in mature inflamed and ischemic brain tissue. PLoS ONE 13(11): e0206597. doi: 10.1371/journal.pone.0206597

PROTOCOL STATUS

Working

GUIDELINES

- use with APAAP (Alkaline Phosphatase - Anti-Alkaline Phosphatase) Kit (Dako K5000, Glostrup, Denmark)
- positive control: human liver

1 See Guidelines

Clear slides

2 Place slides in hybridization oven: 37°C overnight, then 1 hour at 65°C

3 Deparaffination in xylene 3x20 minutes (3 different containers) on a shaker

4 Rehydration in graded ethanol: 3x2 minutes in 100% ethanol, followed by 2x2 minutes in 96% ethanol and at last 2 minutes in 70% ethanol

5 Wash in phosphate buffered saline (PBS) for 5 minutes

Antigen retrieval

6 Antigen retrieval in citrate buffer (10 mM) in a heat steamer for 30 minutes. Afterwards let the slides cool down in phosphate buffered saline for 30 minutes

7 Wash slides in PBS for 5 minutes

Immunostaining

8 Encircle each tissue section with a wax pen

9 Apply the primary antibody in 1% dilution of Bovine serum albumin (BSA) in PBS, 100 µl per section (negative controls: only 1% dilution), incubation overnight in a moisture chamber

10 Tip off excess solution and rinse in demineralized water

11 Wash in PBS for 2x5 minutes

12 Apply the secondary antibody DAKO REAL Link (APAAP Kit, bottle A) 100 µl per section, incubation for 30 minutes in a moisture chamber

13 Tip off excess solution and rinse in demineralized water

14 Wash in PBS for 2x5 minutes

15 Apply Dako REAL APAAP Immunocomplex (APAAP Kit, bottle B), 100 µl per section, incubation for 30 minutes in a moisture chamber

16 Tip off solution and rinse in demineralized water

17 Wash in PBS for 2x5 minutes

18 Prepare the substrate working solution (CHROME): To each 750 µl of AP Substrate Buffer (APAAP Kit , bottle F) add 30 µl chromogen Red 1 (APAAP Kit, bottle C), 30 µl chromogen Red 2 (APAAP Kit, bottle D) and 30 µl chromogen Red 3 (APAAP Kit, bottle D) in this exact order. Mix well and use within 20 minutes!

19 Apply substrate working solution (CHROME), 200 µl per section, incubation for 20 minutes

20 Wash in demineralized water for 2x5 minutes

21 Submerge slides in Mayer´s hematoxylin for 1 minute

22 Rinse in demineralized running water for 5 minutes

Dehydrate and mount slides

23 Dehydrate in graded ethanol: 1 minute in 70% ethanol, following 1 minute in 96% ethanol and 2 minutes in 100% ethanol

24 Place the tissue samples in xylene for 3x1 minute

25 Coverslip using mounting medium