Immunofluorescence staining protocol for co-staining of fetuin-A and GFAP in older human autopsy tissue via Tyramide Signal Amplification

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ABSTRACT

This staining was performed to detect fetuin-A in astrocytes. Fetuin-A and astrocytes were detected in paraffin sections (1 μm thickness) of formalin-fixed human brain tissue. Glial Fibrillary Acid Antigen (GFAP) was marked by a polyclonal rabbit-anti-human antibody (Spring Bioscience Cat# E18320, RRID:AB_1661177, dilution 1:50) and a polyclonal goat-anti-rabbit Alexa Fluor® 488 conjugated secondary antibody (Thermo Fisher Scientific Cat# A-11070, RRID:AB_2534114, dilution 1:300).Fetuin-A was detected by using a monoclonal IgG2a mouse-anti-human antibody (clone MAHS-1, dilution 1.0 μg/mL), raised against purified human fetuin-A in our laboratories. Antibody binding was detected by tyramide signal amplification using a secondary biotinylated polyclonal goat-anti-mouse antibody (Dako Cat# E0433, RRID:AB_2687905, dilution 1:300) and a Tyramide Signal Amplification Kit (Life Technologies, Carlsbad, USA, T-20933). To minimize lipofuscin autofluorescence, sections were counterstained with Sudan Black (Sigma-Aldrich, Munich, Germany, 199664, dilution 0.3% in 70% ethanol, 5 minutes). Nuclei were stained with DAPI (Sigma-Aldrich, Munich, Germany D9542, dilution 0.25 μg/ml, 5 minutes). Sections were mounted with Immumount (Thermo Scientific, Waltham, USA, 9990402) and stored at 8°C in the dark.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

GUIDELINES

use with Tyramide Signal Amplification Kit (life technologies, T-20933)

BEFORE STARTING

Prepare the following components of the Tyramide Signal Amplification Kit according to the manufacturers protocol:

1. Prepare tyramide stock solution by dissolving Component A in 150 μL of Component B (DMSO). (Store unused portions of this stock solution in small aliquots at ≤ – 20°C, protected from light).
2. Prepare a 1% (10 mg/mL) solution of Component D (BSA) in phosphate-buffered saline (PBS).
3. Prepare the horse radish peroxidase (HRP) conjugate stock solution by reconstituting Component C in 200 μl PBS. (This solution may be stored at 2–8°C for up to 3 months).
4. Prepare amplification buffer/0.0015% H2O2 by adding 30% hydrogen peroxide (Component F) to amplification buffer (Component E) to obtain a final concentration of 0.0015% H2O2. For example, add 1 μL of 30% H2O2 to 200 μL of amplification buffer and then add 1 μL of this intermediate dilution (0.15% H2O2) to a further 100 μL of amplification buffer.

See Guidelines

1 Prepare the components of the Tyramide Signal Amplification Kit

Clear slides

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

3 Deparaffination in xylene 3x20 minutes (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 PBS for 5 minutes

Antigen retrieval

6 Antigen retrieval in citrate buffer (10 mM, pH 6) in a heat steamer for 30 minutes. Afterwards let the slides cool down in PBS for 30 minutes

7 Wash in PBS for 5 minutes

Immunostaining

8 Encircle tissue section with wax pen

9 To block non-specific binding, apply a solution of 10% goat serum in PBS, incubation for 1 hour in a moisture chamber

10 Tip off excess solution and apply the primary antibodies MAHS-1 (dilution 1 µg/ml) and Anti-GFAP (dilution 1:50) in 1% BSA in PBS, 100 µl per section (negative controls: only 1% dilution), incubation overnight in a moisture chamber

11 Tip off excess solution and rinse in demineralized water

12 Wash in PBS for 3x5 minutes

13 Apply the secondary antibodies: biotinylated goat-anti-mouse antibody (dilution 1:300) and polyclonal goat-anti-rabbit Alexa Fluor® 488 conjugated secondary antibody (dilution 1:300), both in 1% of BSA in PBS, 100 µl per section, incubation for 90 minutes at room temperature in a moisture chamber.

keep the tissue samples from now on light protected!

14 Tip off excess solution and rinse in demineralized water

15 Wash in PBS for 3x5 minutes

16 Apply the Tyramide Signal Amplification Kit as follows: Dilute the prior prepared HRP solution 1:100 in 1% BSA in PBS, apply 100 µl per section, incubation for 1 hour at room temperature in a moisture chamber.

17 Tip off excess solution and rinse in demineralized water

18 Wash in PBS for 3x5 minutes

19 Prepare a tyramide working solution following the manufacturer's protocol: dilute the prior prepared tyramide stock solution 1:100 in the prepared 0.0015% hydrogen peroxid solution

20 Apply 100 µl per section, incubation for 10 minutes

21 Tip off excess solution and rinse in demineralized water

22 Wash in PBS for 3x5 minutes

23 To minimize lipofuscin autofluorescence, perform counterstaining with Sudan Black (dilution 0.3% in 70% ethanol) for 5 minutes

24 Rinse the container with demineralized water until the solution becomes clear and colorless

25 Wash in demineralized water for 3x5 minutes

26 Counterstain with DAPI (dilution 0.25 µg/ml) for 5 minutes

27 Wash in PBS for 3x5 minutes

28 Coverslip using mounting medium

29 store at 8°C in the dark