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Immunohistochemistry (Stadelmann Lab - single antibody, AP)



Forked from [Immunohistochemistry \(Stadelmann Lab - single antibody, DAB-POX-Avidin-Biotin\)](#)

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Protocol status: Working

We use this protocol and it's working



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Abstract

Staining of FFPE human brain tissue using alkaline phosphatase secondary antibodies.



Paraffin removal and rehydration

- 1 Immunohistochemistry was performed on 2-3 μm thick paraffin sections.
- 2 Remove Excess Paraffin at 56° for 1h-24h 1h
- 3 Rehydration
- 3.1 4x Xylol à 5 min 20m
- 3.2 1x Isopropanol:Xylol 1:1 à 3 min 3m
- 3.3 2× 100% Isopropanol à 3 min 6m
- 3.4 1× 90% Isopropanol à 3 min 3m
- 3.5 1× 70% Isopropanol à 3 min 3m
- 3.6 1× 50% Isopropanol à 3 min 3m
- 3.7 3x wash in aqua dest 1m 30s

Antigen Retrieval

- 4 Antigen Retrieval
- 4.1 heat antigen retrieval (1x citrate buffer 10 mM , pH 6) - 30 min steamer 30m

4.2 3x wash TBS

1m 30s

4.3 formic acid (98%) (for Amyloid beta immunohistochemistry) - 1minute at RT

1m

4.4 3x wash TBS

1m 30s

Blocking

15h 36m 10s

5 3% hydrogen peroxide in TBS 10 min @ RT

10m

6 3x wash TBS

1m 30s

7 Blocking in 10% normal goat serum in TBS for 10 min @ RT

10m

8 Decant 10% NGS in TBS and move directly to the next step without washing

Staining

15h 36m 10s

9 Incubation of primary antibodies over night with respective dilution in 10% NGS @RT in a humid chamber

12h



	target antigen - human	host species	Clone	Company	Catalog Number	Dilution
	beta-Amyloid	mouse	6E10	Zytomed Systems GmbH	Z 932002-Y	1:500

10 3x wash with TBS

1m 30s



- 11 Incubation with secondary antibody coupled to alkaline phosphatase 1:100 at RT for 2h in a humid chamber

2h

	target antigen	host species	Clone	Company	Catalog Number	Dilution
	mouse IgG	goat		Jackson Immuno Research	115-055-003	1:100

- 12 3x wash TBS

1m 30s

- 13 Slides were developed using FastBlue 30-60 min
49 mL TBS pH 7.5, 1 mL Naphtol AS Mix Phosphat, 50 µL Levamisol, 25 mg FastBlue
→ Filtration

1h

- 14 3x wash TBS

1m 30s

Counterstaining + Mounting

15h 36m 10s

- 15 Hematoxylin 10s

10s

- 16 10 min tap water

10m

- 17 Aquatex mounting