Sep 11, 2025

Immunhistochemistry (Stadelmann Lab - single antibody, DAB-POX-Avidin-Biotin)

DOI
https://dx.doi.org/10.17504/protocols.io.8epv5rnw6g1b/v1

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Protocol Citation: Jonas Franz, Katja Schulz, Christine Stadelmann 2025. Immunhistochemistry (Stadelmann Lab - single antibody, DAB-POX-Avidin-Biotin). **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5rnw6g1b/v1

Protocol status: Working
This protocol is established over years and provides robust results.

Created: June 27, 2024

Last Modified: September 11, 2025

Protocol Integer ID: 102505

Keywords: ASAPCRN, primary antibody, single antibody, secondary antibody, coupled secondary antibody, using avidin, immunhistochemistry, avidin, biotin, coupled peroxidase, stadelmann lab

Abstract

This protocol describes staining of any primary antibody using a biotin coupled secondary antibody using Avidin coupled peroxidase.

Paraffin removal and rehydration

14h 35m

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

14h 35m

4 Pretreatment

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

adopt according to tissue and antibody

4.2 3x wash PBS

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

4.4 3x wash PBS

4.5 3% hydrogen peroxide in PBS 10 min @ RT

4.6 3x wash PBS

Blocking

14h 35m

5 Blocking in 10% normal goat serum in PBS for 10 min @ RT

10m

6 Decant 10% NGS in PBS and move directly to the next step without washing

Staining

14h 35m

7 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
	pTau	mouse	at8	Thermo Fisher Scientific	MN1020	1:100

Selected Antibodies

8 3x wash with PBS

1m 30s

9 secondary antibody
Incubation with secondary antibody coupled to biotin at RT for 1h in a humid chamber

1h

	target antigen	host species	Clone	Company	Catalog Number	Dilution
	mouse IgG	sheep		GE Healthcare	RPN1001	1:200

s

10 3x wash PBS

1m 30s

10.1 ExtrAvidin-peroxidase 1:1000 in 10% NGS for 1h in humid chamber at RT

1h

	target	coupled enzyme	Company	Catalog Number	Dilution
	biotin	Peroxidase			1:1000

Counterstaining + Mounting

14h 35m

11 3x wash PBS

1m 30s

12 Slides were developed using DAB (1gin40mL PBS → 1mL)
1 mL Aliquots + 50 mL PBS + 20 µL 30% H2O2)

10m

13 3x PBS

1m 30s

or optional
3x TBS → move to AP coupled secondary staining step 9
(dx.doi.org/10.17504/protocols.io.3byl4952rgo5/v1)

14 10 min tap water

10m

15 Hematoxylin 30s

30s

16 Differentiation dip in HCL 5s

17 Dehydration reverse see **step 3** 40m

18 Entellan mounting with coverslip