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4-HNE immunofluorescence staining

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Protocol status: Working
We use this protocol and it's working in rat liver and kidney tissue as well as pig liver and lung tissue.

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Abstract

This protocol provides an overview of the different steps involved in immunofluorescence staining of 4-hydroxynonenal (4-HNE) using ab46545. This protocol has proven to be effective in rat kidney and liver tissue as well as pig liver and lung tissue.

Materials

- Paraffin tissue section of 5 µm
- Coplin jars (a volume of 250 ml is enough to immerse slides)
- Easy Dip^{TM} container + staining rack
- Xylene
- Ethanol (50% - 100%)
- MiliQ (MQ)
- Citric acid (0,1 M):

	A	B	C	D
	Reagent (concentration)	**Volume needed**		
	Citric acid (anhydrous powder) (192,123 g/mol)	9,60 g	4,80 g	1,92 g
	MQ	500 ml	250 ml	100 ml

- Sodium citrate (0,1 M, pH 6):

	A	B	C	D
	Reagent (concentration)	**Volume needed**		
	Sodium citrate (powder) (258,07 g/mol)	12,91 g	6,46 g	2,58 g
	MQ	500 ml	250 ml	100 ml

Note: Start with a lower volume and adjust to pH. Afterwards, you can fill until the desired volume. Check final pH to be sure.

- Citric acid buffer:

	A	B
	Reagent (concentration)	**Volume needed**
	Citric acid (0,1M)	2,30 ml
	Sodium citrate (0,1M, pH 6)	10,70 ml

	A	B
	MQ	Add until total volume of 130 ml

- Microwave
- Slide container with wet tissues
- Tris NaCl buffer:

	A	B
	Reagent (concentration)	**Volume needed**
	Tris-HCl (0,1 M, pH 7,5) (157,6 g/mol)	7,88 g
	NaCl (0,15 M) (58,44 g/mol)	4,38 g
	MQ	500 ml

- TNT (Tris NaCl, Tween20) buffer:

	A	B
	Reagent (concentration)	**Volume needed**
	Tris NaCl buffer	50 ml
	Tween20 (0,05%)	25 µl

- TNB (Tris NaCl Blocking) buffer:

	A	B
	Reagent (concentration)	**Volume needed**
	Tris NaCl buffer	50 ml
	Bovine Serum Albumin (0,5%)	0,25 g

- Goat serum
- Primary antibody: Rabbit Polyclonal 4-HNE antibody (1/500; Cat.# ab46545; RRID: AB_722490)

- Secondary antibody: Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488 (4 µg/ml; Cat.# A-11008; RRID: AB_143165)
- DAPI (Cat.#62248)
- PBS (1x)
- Coverslips
- Slow Fade Diamond antifade (Fisher Scientific (15,441,244))
- Nail polish

Troubleshooting

Problem

Insufficient antigen retrieval

Solution

Microwave settings might differ between brands/types. Test at which wattage and time combination the antigen retrieval buffer reaches boiling point, without buffer overflowing. Keeping the lid open can help to prevent boiling over. Use this setting to preheat the buffer. Next, find a setting that helps maintain this temperature without the buffer boiling over. This setting can be used for the slides.

Before start

Prepare all buffers and reagents before start of deparaffinization

Deparaffinization

25m

1 Submerge the slides in the following reagents:

1.1 5′ Xylene
5′ Xylene
3′ 100% Ethanol
3′ 90% Ethanol
3′ 70% Ethanol
3′ 50% Ethanol
3′ MQ

25m

Antigen retrieval

42m 30s

2 Fill the Easy Dip™ container with citric acid buffer (±120-130 ml)

3 Preheat the citric acid buffer in the microwave for 1,5′ at 750W (keep the lid of the container open).

1m 30s

4 Immerse the slides in the preheated buffer

5 Place the slides in the microwave for 2 cycles of 4,5′ at 90W with 1-2 minutes in between (keep the lid of the rack elevated).

11m

6 Let the slides cool down for 30′ in the buffer

30m

Incubation with primary antibody

1d 1h 5m

7 Prepare a chamber (slide container) with wet tissues on the bottom to maintain humidity

8 Wash 5′ with TNT

5m

9 Dry slides carefully with tissue paper without affection the sections

10 Block for 1h with TNB + 1/50 normal goat serum 1h

11 Incubate the slides with 1st antibody (TNB + 1/50 normal goat serum + 1/500 4HNE primary antibody) overnight at 4°C 1d

Incubation with secondary antibody 1h 15m

12 Wash 3× 5′ with TNT 15m

13 Dry slides carefully with tissue paper

14 Cover the chamber from light

15 Incubate the slides for 1h with 2nd antibody (TNB + 1/50 normal goat serum + 4 µg/ml 2nd antibody) at room temperature 1h

DAPI nuclear staining 35m

16 Wash 3× 5′ with TNT 15m

17 Dry slides with tissue paper

18 Add DAPI (1/2000 in PBS) for 5′ 5m

19 Dry slides with tissue paper

20 Add DAPI again (1/2000 in PBS) for 5′ 5m

21 Wash 5′ PBS at RT in glass jar or Easy Dip™ container 5m

22 Repeat wash 5′ PBS at RT in glass jar or Easy Dip™ container 5m

Mounting

23 Dry slides with tissue paper

24 Add 1 drop of slow fade in the middle

25 Place a coverslip on top of the slide and remove air bubbles

26 Apply nail polish on the borders to secure the coverslip

27 Store overnight in the dark at room temperature

28 After drying/visualising, the slides can be stored in the fridge or at -20°C

Acknowledgements

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