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Immunofluorescence on human brain FFPE sections to identify neuromelanin in A6, A9, and A10

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ABSTRACT

This is the protocol to scan brain neuromelanin with reduced impact of the chemicals on their original colour and intensity. The immunofluorescence staining protocol is designed to identify human A6, A9, and A10 cell clusters and alpha-synuclein pathology.

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Protocol status: Working
We use this protocol and it's working

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Preparation

1h 10m

1 Select sections, label the staining method on slides, and load slides to slide racks. 1h

2 Check the medium and reagents ready for use. 10m

Deparaffin and rehydration

1h 33m

3 Bake the slides in a 60 °C oven for one hour. 1h

4 Dip slides in HistoChoice for 2 x 7 minutes. 14m

5 Dip slides in 100% ethanol for 2 x 3 minutes. 6m

6 Dip slides in 95% ethanol for 3 minutes. 3m

7 Dip slides in 70 % ethanol for 3 minutes. 3m

8 Dip slides in MQ water for 3 minutes. 3m

9 Dip slides in 1XPBS. 1m
Note: The slides can be mounted with DAKO fluorescence mounting medium (Agilent, cat# S302380-2) for brightfield scanning by VS200 to acquire neuromelanin images, then de-coverslip in 1XPBS to proceed for the following antigen retrieval step.

Heat-induced Antigen retrieval 2h 6m

10 Transfer slides into the slides chamber filled with 1X citric buffer (CB) pH6.0, and apply HIAR using a programmable antigen retrieval cooker (Aptum Bio Retriever 2100, Aptum Biologics Ltd, UK) at a peak temperature of 121 °C, followed by the gradual cooling procedure for ~2 hours. 2h

11 Dip slides in MQ water for 1 minute. 1m

12 Dip slides in 1XPBS for 5minutes 5m

Quench aldehyde group

50m

13 Prepare NaBH4 (*Sigma #72320, stored in the toxic cabinet)* at a final concentration of 0.5% (w/v) in 1X PB 10m
Make this fresh in the fume hood!

14 Transfer slides into this quenching buffer for 30 minutes (on ice). 30m

15 Slides undergone PBS washing 2 X 5 minutes. 10m

Suppressing lipofuscin autofluorescence

43m

16 Immersing slides in 70% ethanol for ~1 minute. 1m

17 Transfer the slides into 0.1% SBB (in 70% ethanol) for ~30 minutes. 30m

18 Wash slide with 70% ethanol for ~1 minute (dip several times) 1m

19 Rinsing slides with pure H_2O briefly. 1m

20 Wash slides in 1XPBS 2 X 5 minutes. 10m

Blocking buffer treatment 2h

21 Set up the StainTray slide staining system (Sigma, Z670146) or incubation boxes. Circle the samples with PAP pen. 30m

22 Blocking with Human Fc blocker: Human FC blocker (BD Pharmingen Human BD Fc Block, clone Fc1, #564220) in 1XPBS 1:200 @ Room temperature for 30 minutes. 30m

23 Blocking with IF buffer @ Room temperature for 60 minutes. 1h

Primary antibody incubation for indirect labeling 12h 20m

24 Make a Primary antibody cocktail in IF buffer. 20m

	A	B
	CalB1(Ch)	Antibodies.com #A85369
	ALDH1A1 (Gt)	R&D AF5869

Primary Antibodies

25 Incubate sections with primary antibody cocktail in the CoolRm @ 4 °C overnight. 12h

Secondary antibodies incubation (from this step, protect from light)

2h 30m

26 Wash sections with 1xPBS 3X10 minutes

30m

27 Make the 2nd antibody cocktails as above in IF blocking buffer @ Room temperature for 120 minutes.

2h

	A	B	C	D
	Cat#	ThermoFisher SA5-10091	Sigma SAB4600031	Sigma B2261
	Reagent	Dn@Gt-DL755	Dn@Ch IgY-CF488A	Hoechst 33,342 (1mg/ml stock)
	Dilution	1:200	1:250	1:1000

Reagent list

Direct labelling with conjugated antibodies

2h 30m

28 Wash sections in 1XPBS 3X5 minutes.

15m

29 Dilute conjugated antibodies into IF blocking buffer as below @ Room temperature for 120 minutes.

2h

	A	B	C
	Conjugated Abs	**TH- AF647**	**S129-AF568**
	Cat#	BioLegend #818008	Abcam Ab307166
	Dilution	1:100	1:100

Conjugated antibodies

30 Wash sections in 1XPBS 3X5 minutes.

15m

Coverslip

1h 30m

31 Leave sections at dark for drying for about 5~10 minutes or dry all the solution residue on the sections, mounting with anti-fade media (DAKO Fluorescence Mounting Medium), and leave @ Room temperature for >30 minutes. 1h

32 Seal the corners of the coverslips with nail polish. 30m

Storage before scan

10m

33 Store slides in the slide box and store in the fridge or cool Rm. 10m

34 Ready for VS200 scanning.

Appendix of medium

35 Home-made IF Blocking buffer (NDS):

- 2% Donkey serum (Sigma, D9663)
- 1% BSA (best with IgG-free and protease-free) (Sigma, A9085 or JIR #001-000-173).
- 0.2% TritonX-100 (Sigma, T9284).
- 0.1% gelatine (from fish skin, Sigma, G7041).
- 0.1% Tween-20 (Sigma, P1379).
- 0.01% Sodium Azide (Sigma, S2002)

in 1XPBS, aliquoted and stored @ -20 °C.