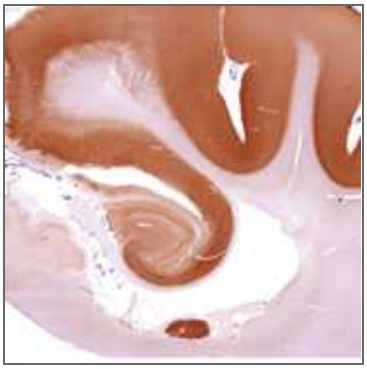

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Protocol status: Working
We use this protocol and it's working

Created: Aug 07, 2023

Automated Immunohistochemistry Staining

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ABSTRACT

The protocols describes the steps for automated immunohistochemistry staining using the Ventana Discovery® XT
Immunostainer.

MATERIALS

- Primary AT8 antibody (Thermo Fisher Scientific, MN1020)
- Antibody diluent solution (Ventana)
- Ventana Discovery® XT Immunostainer: https://www.medicalexpo.com/prod/roche/product-71020-527513.html
- Leica CV5030 automated slide cover-slipping machine
- 100 % ethanol
- Oven

Last Modified: Dec 07, 2023

PROTOCOL integer ID: 86041

Keywords: ASAPCRN, Immunohistochemistry, Brain sections, Ventana Discovery® XT Immunostainer, Antibody diluent solution (Ventana)

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Grant ID: ASAP-000478

Preparation

1h

1 Heat tissue dry tissue sections for 01:00:00 at 60 °C

1h

2 Generate tissue sections using standard microtome sectioning protocols.

3 Prepare primary and secondary antibody solutions as per manufacturer's protocol - see below for example for AT8.

4 Primary AT8 antibody (Thermo Fisher Scientific, MN1020) diluted at 1:100 in antibody diluent solution (Ventana). Secondary anti-mouse antibody (Abcam) diluted at 1:100 in antibody diluent solution (Ventana).

5 Perform immunohistochemical staining using the Ventana Discovery® XT Immunostainer – following manufacturers guidelines.

6 Print barcoded slide labels corresponding to the correct protocol on the Ventana machine and stick them to the top of each slide following the slide heating.

Processing Slides Using the Ventana Machine

25m

7 Place slides into the Ventana machine and ensure all bulk reagents are sufficiently filled

8 Begin the staining protocol.

9 Upon completion of the staining, activate the counterstaining step.

10 Following counterstaining, remove the slides and wash in soapy water 5x.

11 Leave slides in running water for 00:05:00 mins.

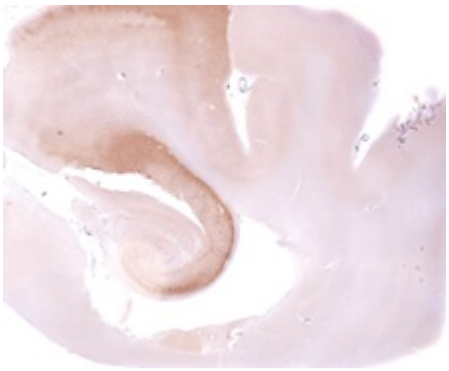
5m

12 To deparaffinise sections, place slides in 100 % ethanol for 00:05:00 mins, remove and place in 20m separate 100 % ethanol for 00:05:00 mins. Remove from ethanol and place in xylene for 00:05:00 mins, remove and place in separate xylene for 00:05:00 mins

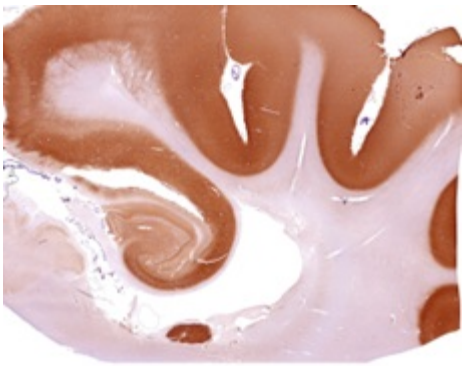
13 For cover-slipping of slides following deparaffinisation – use the Leica CV5030 automated slide cover-slipping machine.

14 Stained slides digitised on a NanoZoomer S360 scanner (Hamamatsu) – Brightfield scan profile at x40 magnification.

15 Upload digitised slides to NZ Connect (1.0.36 (IVD)) slide viewing platform.



AT8 staining



Alpha-synuclein staining