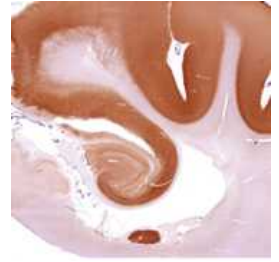


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Automated Immunohistochemistry Staining

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Toby J Curless^{1,2}, Hemanth Ramesh Nelvagal^{1,2}, Zane Jaunmuktane^{1,3,2}

¹Department of Clinical and Movement Neurosciences, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK;

²Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20 815, USA;

³Queen Square Brain Bank for Neurological Disorders (QSBB), 1 Wakefield Street, London WC1N 1PJ, UK



Toby J Curless

Department of Clinical and Movement Neurosciences, UCL Insti...

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Protocol status: Working

We use this protocol and it's working

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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

Troubleshooting

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

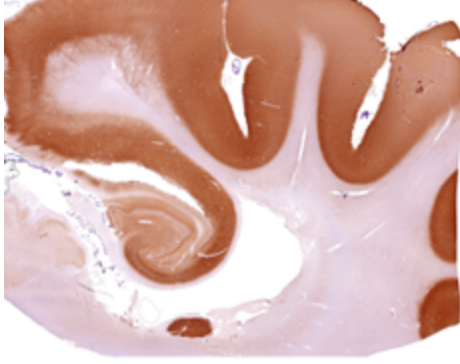
25m

- 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 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 20m
- 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