Immunohistochemistry – Novolink Polymer Detection Systems

Judi O'Shaughnessy[1], Dr Jenna Gregory[2]

[1]Research Assissant, University of Edinburgh; [2]SCREDS Clinical Lecturer, University of Aberdeen

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Judi O'Shaughnessy

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ABSTRACT

Immunohistochemistry is a method used to detect specific antigens in tissue sections. It exploits the principal of antibodies binding to specific antigens (epitopes). Visualisation of the target antigen is achieved by attaching an enzyme to the antibody-antigen conjugate and visualising it with (in this case) DAB chromogen.

This SOP applies to FFPE tissue sections cut at 4µm. We use it almost exclusivley for human brain tissue however, it can work well for other tissue types. Our SOP is adapted from the Leica Novolink Polymer Detection Systems user manual, product number RE7280-K.

We have included our ticksheet, we find it helps to keep us on track.

IHC_SOP_v3.0.pdf

IHC_TS_v2.0.pdf

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MATERIALS TEXT

Materials_IHC.pdf

BEFORE STARTING

- In this protocol, 'alcohol' refers to IMS. Ethanol can be used instead but it isn't necessary.

-Consider what pretreatments you need and how long they might take before undertaking starting.

-Get the Novolink kit from the fridge to allow it enough time to get up to room temperature.

-Make sure you have made up enough wash buffer.

Before Starting

1 - Purpose:
The purpose of this SOP is to outline the correct procedures for performing Immunohistochemistry (IHC) using the Novolink Polymer Detection System kit. It is adapted from the Novolink Polymer Detection Systems instruction manual and is intended for the use by all staff, students, and users of the Gregory Laboratory.

- Safety First:
Before starting, you must read all relevant Health & Safety documentation, and fully read this SOP. Please speak to any of the Gregory Lab technical staff if there is anything you are unsure about before embarking on your experiment, we are always happy to help.

- COSHH and Risk assessments
- SOP

- Special note:
See appendix A for methods for making up solutions.
See appendix B for a list of commonly used antibody dilutions, pre-treatments and ordering information.

Prepare treatment materials

2 * Get Novolink kit from the fridge, bring to room temperature
* Make up 1:10 TBS (tris:saline)
* If using, make up citric acid buffer (see step 3)
* Get sequenza racks and coverplates

Deparaffinise FFPE sections and remove formalin pigment

3 Put your slides in a staining rack and take them through a series of xylene and alcohol baths (see below). Briefly agitate the rack when placing it in each bath and drain well before moving onto the next one.

Xylene

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3 min

Xylene
3 min

Alcohol
2 min

Alcohol
2 min

Saturated Alcoholic Picric acid
15 min

Wash in warm, running tap water
15 min

Antigen retrieval

4 It is always a good idea to refer to the datasheet provided with the antibody for up to date information regarding pre-treatments and dilutions, different antibodies require different conditions. Appendix B provides a quick reference guide to commonly used antibodies.
The pre-treatment most often used in The Gregory Lab is heat induced epitope retrieval with citric acid buffer (pH6), you will need to make up a working solution (see appendix A for methods for making solutions).

* For 10mM (working) solution dilute 1:10.
E.g.,50ml of 100mM citric acid buffer and 450ml of dH2O.

Using the pressure cooker

5 The settings, times and temperature on your pressure cooker may be different to the one used in the Gregory Lab. The instructions below are optimised for a 3L Drew and Cole 'Pressure King Pro' domestic pressure cooker.

Put 500ml dH2O in the basin of the pressure cooker.
Place slides in a slide rack, pop the rack in a suitable pressure cooker pot and pour in antigen retrieval buffer (e.g., citric acid or tris/EDTA). Make sure the slides are fully covered.
Put the lid on and lock it by turning it anticlockwise until you hear a click.
Turn the pressure release valve to the closed position.

Press the 'browning/meat' button and use the + and – buttons to adjust the time to 5 min (if it starts before you're ready, press cancel and try again). After a short delay, the pressure cooker will start automatically. The full cycle takes around 20 min.
When the cycle has finished there will be a short beep sound and the display will read 'end', press the cancel button. You can wait for the pressure to release naturally or you can move the valve to the open position to release the pressure more quickly.
Beware! The slides and pressure cooker will be very hot and should be handled with extreme

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caution. Remove the lid of the pressure cooker, lift the basin out and place it in the sink. Cool the slides down in running tap water.

Coverplating

6 Use dH2O to fit coverplates to your slides. Make sure there are no bubbles present and press securely into the sequenza rack.
Fill the wells to the top with dH2O, leave for 5 min. If the water flows through too quickly, or not at all, remove the coverplate from the slide and try again.

Using Novolink Polymer Detection kit

7 Apply the following Novolink Polymer Detection kit reagents. See below for how to make working primary antibody and DAB solutions.

Peroxidase block (3 drops)
30 min

TBS
5 min

Protein block (3 drops)
15 min

TBS
5 min

Primary antibody
30 min

TBS
5 min

Post primary block (2 drops)
30 min

TBS
5 min

Novolink Polymer (2 drops)
30 min

TBS
5 min

DAB
5 min

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dH2O
5 min

Making up primary antibody

8 Every antibody is different. Check the data sheet that came with your antibody for the concentration and recommended working dilution. Each slide requires 100µl of antibody solution and is diluted with wash buffer, in the Gregory Lab we generally use TBS.

When making up antibody we need to know
° What is the volume of solution required?
° What is the dilution?
° How much antibody will be needed?

DAB Chromogen

9 The ratio of DAB chromogen to DAB substrate buffer is 50µl:1000µl. This is plenty of solution for 10 slides.
Pipette 100µl of DAB solution into the well of each coverplate, leave for 5 min.

Fill up wells with dH2O, leave for 5 min.

Remove coverplates and Counterstain Slides

10 Carefully remove each slide with their attached cover plate from the sequenza, gently lift the slide off the coverplate (remembering to lift rather than slide them off).

Put the slides in a slide rack and wash well in running tap water for 5 min.

Counterstain your slides in the below solutions. As with dewaxing, briefly agitate the rack at each stage and drain well before moving into the next reagent

Haematoxylin
2 min

Wash in running tap water
1 min

Lithium carbonate
30 sec

Wash in running tap water
1 min

Dehydrate, clear and Mount.

11 Drain off as much water as possible from the rack, then fully dehydrate the slides by taking them through a series of alcohols.

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In each alcohol bath, agitate the slides for 30-60 seconds or until they are 'clear'. You will see no water steaks and the glass will appear solid. Alternatively, agitate the rack briefly and leave for two minutes.

Drain the rack very well before moving onto the next alcohol.

70% Alcohol
100% Alcohol
100% Alcohol

Using the same technique as for the alcohols, clear the slides in at least two changes of xylene.
When the slides are clear of alcohol there will be no streaks and they will 'disappear' when submerged in xylene.

Your slides are now ready to be coverslipped.

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