

Immunohistochemistry – Novolink Polymer Detection Systems

Things to do while you're waiting for your timer

- ✓ Check DPX and put a pipette in ready for later
- ✓ Get the kit from the cold room

- ✓ Do you need to make antigen retrieval solution?
Check you have enough.
- ✓ If using, dilute citric acid retrieval 1:10 (stock: dH₂O)

- ✓ Make up 1:10 TBS (Tris:saline)
Tris is in the fridge, saline is in the cupboard

- ✓ Make up DAB
50µl chromogen + 1ml Dab substrate buffer

The steps

Deparaffinise slides

Xylene	3 min	<input type="checkbox"/>
Xylene	3 min	<input type="checkbox"/>
Alcohol	2 min	<input type="checkbox"/>
Alcohol	2 min	<input type="checkbox"/>
Do not wash in water		

Remove Formalin Pigment

Saturated Alcoholic Picric acid	15 min	<input type="checkbox"/>
Wash in warm, running tap water	15 min	<input type="checkbox"/>

Antigen retrieval

Put 500ml dH ₂ O in the pressure cooker before starting	40 min	<input type="checkbox"/>
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Coverplate

Drops

dH ₂ O	5 min	<input type="checkbox"/>
Peroxidase block (3 drops)	30 min	<input type="checkbox"/>
TBS	5 min	<input type="checkbox"/>
Protein block (3 drops)	15 min	<input type="checkbox"/>
Make up antibody (antibody:TBS)		<input type="checkbox"/>
TBS	5 min	<input type="checkbox"/>
Primary antibody	30 min	<input type="checkbox"/>
TBS	5 min	<input type="checkbox"/>
Post primary block (2 drops)	30 min	<input type="checkbox"/>
TBS	5 min	<input type="checkbox"/>
Novolink Polymer (2 drops)	30 min	<input type="checkbox"/>
TBS	5 min	<input type="checkbox"/>
DAB	5 min	<input type="checkbox"/>
dH ₂ O	5 min	<input type="checkbox"/>
Wash well in running water		<input type="checkbox"/>
Counterstain with haematoxylin		<input type="checkbox"/>
Blue in lithium carbonate		<input type="checkbox"/>
Dehydrate, clear and mount		<input type="checkbox"/>