Day1: Immunohistochemistry – Antibody staining (Novolink Polymer Detection kit)

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: dH_2O)

✓ 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**	☐
Xylene	**3 min**	☐
Alcohol	**2 min**	☐
Alcohol	**2 min**	☐

Place slides in tap water (but see below.

NB: Do not place slides in tap water if employing optional picric acid step below

(Optional step) Remove Formalin Pigment

Saturated Alcoholic Picric acid	**15 min**	☐
Wash in warm, running tap water	**15 min**	☐

Antigen retrieval **15 min** ☐

Put 500ml dH_2O in the pressure cooker before starting

Coverplate ☐

Drops

dH_2O	**5 min**	☐
Peroxidase block (3 drops)	**30 min**	☐
Wash with TBS	**5 min**	☐
Protein block (3 drops)	**15 min**	☐
Make up antibody (antibody:TBS)		☐
Wash with TBS	**5 min**	☐
Primary antibody	**30 min**	☐
Wash with TBS	**5 min**	☐
Post primary block (2 drops)	**30 min**	☐
Wash with TBS	**5 min**	☐
Novolink Polymer (2 drops)	**30 min**	☐
Wash with TBS	**5 min**	☐
DAB	**5 min**	☐
dH_2O at room temp	**Overnight**	☐

Day 2: Immunohistochemistry – TDP-43 Aptamer

Things to do while you're waiting for your timer	The steps		
	Antigen retrieval (citric acid pH6)	**15 min**	☐
	Coverplate		☐
	dH_2O	**5 min**	☐
	Peroxidase block (3 drops)	**30 min**	☐
	Wash with TBS	**5 min**	☐
	Avidin block (4 drops)	**15 min**	☐
	Wash with TBS	**5 min**	☐
	Biotin Block (4 drops)	**15 min**	☐
	Wash with TBS	**5 min**	☐
	Make up aptamer in MilliQ H_2O		☐
	Wash with dH_2O	**5 min**	☐
	Aptamer incubation (1in500)	**3 hours**	☐
	Fix step 4% PFA in dH_20	**Overnight**	☐

Day3: Immunohistochemistry – TDP-43 Aptamer

	Re-coverplate		
	Wash with dH_2O	**5 min**	☐
✓ Make up Fast Red Chromogen (1:60)	**Anti-Biotin/Alk Phos 1:200 in MilliQ**	**30 min**	☐
1 µl Fast Red + 59 µl Fast Red substrate buffer So ... 17 µl chromogen + 983 µl Fast Red substrate buffer	Wash with dH_2O	**5 min**	☐
	Fast Red (1:60)	**5 min**	☐
	Wash with dH_2O	**5 min**	☐
	Wash well in running tap water		☐
	Counterstain with haematoxylin		☐
	Blue in lithium carbonate		☐
	Dehydrate, clear and mount		☐

References for citation of this method

To cite this dual staining method, please cite both Rifai et al., 2024 and Waldron et al., 2024 (this SOP) below:

The citation, Rifai *et al*., 2024, for the first publication employing dual staining for *NEK1* and TDP-43 in *Brain Pathology*:

Clinicopathological analysis of *NEK1* variants in amyotrophic lateral sclerosis. Olivia M. Rifai, Fergal M. Waldron, Danah Sleibi, Judi O'Shaughnessy, Danielle J. Leighton*, Jenna M. Gregory*‡ (2024). *Brain Pathology* doi: 10.1111/bpa.13287 (in press at the time of publication of this SOP) *equal contributions, ‡corresponding author

The citation, Waldron *et al*., 2024, for this SOP published on *protocols.io* is:

Antibody and TDP-43 RNA aptamer dual staining to detect patterns of co-pathology in FFPE-preserved human tissue, as described in Rifai et al., 2024 (Brain Pathology): An SOP and tick-sheet. Fergal M. Waldron, Olivia M. Rifai, Jenna M. Gregory‡, (2024). Protocols.io 2024; DOI: dx.doi.org/10.17504/protocols.io.14egn6wnml5d/v1, ‡corresponding author.