Day 1: Immunohistochemistry – TDP-43 Aptamer

Things to do while you're waiting for your timer

✓ Get the blocking reagents from the cold room

✓ Make up 10mM citric acid retrieval agent 1:10 from stock.
Stock: 100 mM citric acid (19.12g in 1L dH_2O).

✓ Make up 1:10 TBS (Tris:saline)

✓ If using, dilute citric acid retrieval 1:10 (stock: dH_2O)

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 ☐

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 in fridge**	☐

Day 2: Immunohistochemistry – TDP-43 Aptamer

	Re-coverplate (optional)		
	Wash with dH_2O	**5 min**	☐
✓ Make up DAB Chromogen (1:20)	**Anti-Biotin/HRP 1:100 in MilliQ**	**30 min**	☐
1 µl DAB chromogen + 19 µl DAB substrate buffer So ... 50 µl DAB chromogen + 950 µl DAB substrate buffer	Wash with dH_2O	**5 min**	☐
	DAB (1:20)	**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

Please cite both of these if using this method:

The citation, Spence and Waldron *et al*., 2024, for the first publication for the development, modification and employment of the TDP-43 RNA aptamer to stain human tissue published in *Acta Neuropathologica*.

> **RNA aptamer reveals nuclear TDP-43 pathology is an early aggregation event that coincides with STMN-2 cryptic splicing and precedes clinical manifestation in ALS.**
> Holly Spence*, Fergal M. Waldron*, Rebecca S. Saleeb, Anna-Leigh Brown, Olivia M. Rifai, Martina Gilodi, Fiona Read, Kristine Roberts, Gillian Milne, Debbie Wilkinson, Judi O'Shaughnessy, Annalisa Pastore, Pietro Fratta, Neil Shneider, Gian Gaetano Tartaglia, Elsa Zacco, Mathew H. Horrocks, Jenna M. Gregory‡. 2024. *Acta Neuropathologica* 2024 Mar 5;147(1):50. DOI: 10.1007/s00401-024-02705-1. *equal contributions, ‡corresponding author.

The citation for the SOP and tick-sheet, Waldron and Spence *et al*., 2024, published on *protocols.io* is:

> **TDP-43 RNA aptamer staining to detect pathological TDP-43 in FFPE human tissue, as described in Spence and Waldron et al., 2024 (*Acta Neuropathologica*): A SOP and tick-sheet. v2.**
> Fergal M. Waldron*, Holly Spence*, Jenna M. Gregory‡. *protocols.io* 2024; DOI: dx.doi.org/10.17504/protocols.io.eq2lyjo4mlx9/v2. *equal contributions, ‡corresponding author.