



### Immunohistochemistry – TDP-43 Aptamer

#### Things to do while you're waiting for your timer

- ✓ Get the blocking reagents from the cold room
- ✓ Do you need to make antigen retrieval solution?  
Check you have enough.
- ✓ Make up 10mM citric acid retrieval agent 1:10 from stock. Stock: 100 mM citric acid (19.12g in 1L dH<sub>2</sub>O)
- ✓ Make up 1:10 TBS (Tris:saline)

#### 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"/>
Picric acid incubation	15 min	<input type="checkbox"/>
Wash in water	5 min	<input type="checkbox"/>

##### **Antigen retrieval (citric acid pH6)**

15 min

##### **Coverplate**

dH<sub>2</sub>O 5 min

Peroxidase block (3 drops) 30 min

TBS 5 min

Avidin block (4 drops) 15 min

1<sup>st</sup> Wash with TBS 5 min

2<sup>nd</sup> Wash with TBS 5 min

Biotin Block (4 drops) 15 min

1<sup>st</sup> Wash with TBS 5 min

2<sup>nd</sup> Wash with TBS 5 min

Make up aptamer in MilliQ H<sub>2</sub>O

Wash with dH<sub>2</sub>O 5 min

Aptamer incubation (1in500) 3 hours

Fix step 4% PFA in dH<sub>2</sub>O Overnight

Wash with dH<sub>2</sub>O 5 min

Anti-Biotin/HRP 1:100 in MilliQ 30 min

- ✓ Make up DAB

50µl chromogen + 1ml Dab substrate buffer

Wash with dH<sub>2</sub>O 5 min

DAB 5 min

Wash with dH<sub>2</sub>O 5 min

Wash well in running tap water

Counterstain with haematoxylin

Blue in lithium carbonate

Dehydrate, clear and mount

#### Reference

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](#)<sup>†</sup>. 2024. *Acta Neuropathologica* (in press at the time of release of this TS on 01/03/2024) \*equal contributions, <sup>†</sup>[corresponding author](#).