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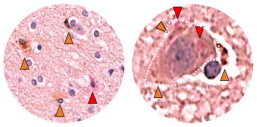
An adaptable protocol for antibody & TDP-43 RNA aptamer dual immunohistochemical staining for FFPE-preserved human tissue: with SOP and tick-sheet.



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Fergal M Waldron[1], Jenna Gregory[1]

[1]University of Aberdeen

Fergal M Waldron
University of Aberdeen

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We use this protocol and it's working

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Abstract

Here we provide a protocol with SOP and tick-sheet, for correct procedures to dual stain FFPE human tissue for, 1) pathological TDP-43 protein, along with 2) any other protein of interest, with the aim of visualising protein of interest histology in the context of TDP-43 pathology.

This method utilises a published TDP-43 RNA aptamer (biotinylated)(Spence and Waldron, 2024 in *Acta Neuropathologica* here https://link.springer.com/article/10.1007/s00401-024-02705-1) which detects pathological TDP-43 with greater sensitively and specificity than with current antibody approaches.

This protocol can be implemented for dual staining of any rabbit or mouse primary detection antibody (using the Novolink Polymer Detection System), and with our TDP-43 RNA aptamer (Spence and Waldron, 2024)

The resulting dual stain will show brown chromogen (detecting the target of the primary antibody) with red chromogen(detecting TDP-43 pathology with our TDP-43 RNA aptamer).

Reference for citation of this method

To cite this dual staining method, please cite Waldron and Gregory, 2024 (here) published on protocols.io:

An adaptable protocol for antibody & TDP-43 RNA aptamer dual immunohistochemical staining for FFPE-preserved human tissue: A SOP and tick-sheet.
Fergal M. Waldron‡, Jenna M. Gregory‡, (2024). Protocols.io 2024;
DOI: dx.doi.org/10.17504/protocols.io.yxmvm97k6l3p/v1
‡equal contributions and co-corresponding authors

Attachments

PDF
Adaptable_IHC_SOP_Do...
392KB

PDF
Adaptable_IHC_TS_Dou...
229KB

Image Attribution

Jenna Gregory

Guidelines

For users attention

Sequenza immunostaining racks: This protocol is designed for use with Sequenza immunostaining racks

Essential fixation step (end of day 2): The TDP-43 RNA aptamer published in Zacco *et al.*, 2022 was designed for flexible use, with moderate binding affinity (e.g. as desirable for some single molecule imaging applications). To improve TDP-43 RNA aptamer binding for staining human tissue, Spence and Waldron *et al.*, 2024 employ an overnight fixation step after incubation with TDP-43 RNA aptamer. This fixation step is essential for reliable and reproducible tissue staining with TDP-43 RNA aptamer. Without the fixation step, the aptamer will not bind with a high enough affinity resulting in partial staining with potential to lead to misleading and erroneous pathological conclusions.

Steric hindrance occurs when molecules (here, antibody and aptamer) affect their respective binding to their target antigens, with the potential for reduced or absence of detectable fluorescence signals.

Therefore , it is imperative that users first optimise single staining protocols for both their primary antibody (mouse or rabbit only for compatibility with the Novolink Polymer Detection System), and for TDP-43^{Apt}, before attempting the dual stain outlined here.

Materials

Biological materials

- FFPE tissue slides

Reagents

Histology
- Xylene
- Ethanol
- Haematoxylin
- Lithium carbonate
- DPX mountant (or similar)

Immunohistochemistry
- Citric acid
  - 5N hydrochloric acid & 5N sodium hydroxide to bring citric acid to pH 6
- MilliQ H2O
- Distilled H20
- Novolink Polymer Detection System Kit (Leica Biosystems; ???)
- Peroxidase block (3% H2O2) – included in Novolink Polymer Detection Kit
- TBS
- Avidin/Biotin Block: e.g. Avidin/Biotin Blocking Kit (Abcam, ab64212)
- TDP-43 Aptamer (TDP-43APT): The sequence is: CGGUGUUGCU with a 3' Biotin-TEG modification, purified using HPLC, scale: 1.0 µM synthesis (as published in Nature Communications here doi: 10.1038/s41467-022-30944-x by Zacco et al., 2022).
- 4% w/v Formaldehyde: from e.g. Pierce™ 16% Formaldehyde (w/v), Methanol-free (ThermoFisher Scientific, Cat No. 28906
- Anti-Biotin–Alkaline Phosphatase antibody produced in goat (Abcam; ab6652)
- Fast Red Chromogen (available from Leica BioSystems)

Equipment

Histology
- Fume hood (for use with Xylene)
- Slides and coverslips
- Histology staining racks and pots
- Coplan jars or similar

Immunohistochemistry
- pH meter (to make citric acid up to pH 6)

- Sequenza rack and coverplates
- Pressure cooker (for antigen retrieval): e.g. Drew and Cole Pressure King Pro
- Refrigerator
- Plastics and other (Pipettes and tips, Falcon tube, dropper, eppendorfs)

Troubleshooting

Safety warnings

Safety First
Before starting, please seek out and read all relevant Health & Safety documentation, and fully read this SOP.

Before working please make sure these have been carried out.
> COSHH assessment
> Risk assessment
> Safe System of Work
> SOP read and understood

Before start

See Appendix A for materials, Appendix B solution recipes, and Appendix C for pressure cooker instructions.

Protocol references

Reference for citation of this method

To cite this dual staining method, please cite Waldron and Gregory, 2024 (here) published on protocols.io:

An adaptable protocol for antibody & TDP-43 RNA aptamer dual immunohistochemical staining for FFPE-preserved human tissue: A SOP and tick-sheet.
Fergal M. Waldron*, Jenna M. Gregory* (2024). *Protocols.io* 2024;
DOI: dx.doi.org/10.17504/protocols.io.yxmvm97k6l3p/v1
*equal contributions and co-corresponding authors

Other relevant publications

The citation, Spence and Waldron *et al.*, 2024, for detection of pathological TDP-43 in human tissue using the TDP-43 RNA aptamer (TDP-43^{APT}) 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, Zacco *et al.*, 2022, for design of the TDP-43 RNA aptamer (TDP-43^{APT}) to visualize TDP-43 condensates with super-resolution microscopy published in *Nature Communications*:

Probing TDP-43 condensation using an in silico designed aptamer.
Elsa Zacco, Owen Kantelberg, Edoardo Milanetti, Alexandros Armaos, Francesco Paolo Panei, Jenna M. Gregory, Kiani Jeacock, David J. Clarke, Siddharthan Chandran, Giancarlo Ruocco, Stefano Gustincich, Mathew H Horrocks, Annalisa Pastore, Gian Gaetano Tartaglia (2022). Nature Communication 2022 Jun 23;13(1):3306. doi: 10.1038/s41467-022-30944-x.

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
*equalcontributions, ‡corresponding author

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