The ability to detect proteins by Western blot, particularly from tissue samples, can be hampered by the choice of membrane and whether a fixation step is used. Here we compare three transfer membranes, polyvinylidene difluoride (PVDF) 0.2 μm, PVDF 0.45 μm and nitrocellulose 0.45 μm for the detection of rat α-synuclein from tissue and recombinant human tau. For α-synuclein fixation of the membrane directly after transfer was imperative for detection of the protein and use of the PVDF 0.45 μm membrane gave the highest detection signal. For tau, the signal was highest on nitrocellulose 0.45 μm and fixation did not enhance the signal.
MATERIALS

STEP MATERIALS

- NuPAGE® trade; LDS Sample Buffer (4X) Thermo Fisher Catalog #NP0008
- NuPAGE® trade; 4-12% Bis-Tris Protein Gels, 1.5 mm, 15-well Thermo Fisher Catalog #NP0336PK2
- NuPAGE® trade; MES SDS Running Buffer (20X) Thermo Fisher Catalog #NP0002
- Immobilon-PSQ PVDF Membrane 0.2um roll Millipore Sigma Catalog #ISEQ00005
- Immobilon-P PVDF Membrane, 0.45um, roll Millipore Sigma Catalog #IPVH00010
- Nitrocellulose Transfer Membrane Amersham Biosciences Catalog #10600002
- Methanol Contributed by users
- NuPAGE™ transfer buffer Thermo Fisher Scientific Catalog #NP0006
- PBS Contributed by users
- Tween-20 Sigma Aldrich Catalog #P9416
- Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab Contributed by users Catalog #GENA934-1ML
- SuperSignal® trade; West Pico PLUS Chemiluminescent Substrate Thermo Fisher Catalog #34579
- Polyclonal Rabbit Anti-Human Tau Unconjugated Ig fraction Agilent Technologies Catalog #A002401-2
- α-Synuclein (D37A6) XP® Rabbit mAb Cell Signaling Technology Catalog #4179
- Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab Contributed by users Catalog #GENA934-1ML
PROTOCOL MATERIALS

- Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab Contributed by users Catalog #GENA934-1ML

- NuPAGE™ transfer buffer Thermo Fisher Scientific Catalog #NP0006

- PBS Contributed by users

- Tween-20 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9416

- Nitrocellulose Transfer Membrane Amersham plc Catalog #10600002

- NuPAGE&trade; MES SDS Running Buffer (20X) Thermo Fisher Catalog #NP0002

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- Methanol Contributed by users

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SAFETY WARNINGS

1. Follow safety guidelines and wear correct PPE when handling fixation solution containing 4% paraformaldehyde and 0.1% gluteraldehyde.

Protein samples were boiled for 3 minutes in 1x NuPAGE® LDS Sample Buffer (4X) Sigma Aldrich Catalog #NP0008 and ran on NuPAGE®trade; 4-12% Bis-Tris Protein Gels, 1.5 mm, 15-well Sigma Aldrich Catalog #NP0336PK2 in NuPAGE®trade; MES SDS Running Buffer (20X) Sigma Aldrich Catalog #NP0002 using the XCell SureLock Mini-Cell Electrophoresis System

Equipment

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at 200 V for 30 minutes.

2. Three membranes were selected to test Western blot transfer and detection, PVDF 0.2 μm Immobilon-PSQ PVDF Membrane 0.2um roll Sigma Aldrich Catalog #ISEQ00005 PVDF 0.45 μm Immobilon-P PVDF Membrane, 0.45um, roll Sigma Aldrich Catalog #IPVH00010 and nitrocellulose 0.45 μm Nitrocellulose Transfer Membrane Sigma Aldrich Catalog #10600002.

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PVDF membranes were first activated with methanol by incubating for 3 minutes. Nitrocellulose membranes do not need to be activated first.

The gel was transferred onto each membrane using the

Equipment

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with

- [NuPAGE™ transfer buffer](https://www.sigmaaldrich.com/nupage-transfer-buffer.html) Sigma Aldrich Catalog #NP0006 + 20%
- [Methanol](https://www.sigmaaldrich.com/methanol.html) Sigma Aldrich

at 30 V for 1 hour. The blot module was kept on ice and surrounded by ice to keep cool and prevent protein damage.

Safety information

PFA and gluteraldehyde are toxic and should be handled in a chemical hood with appropriate PPE. The fixation solution should never be disposed of down the sink, but disposed of in a separate container for disposal through correct waste systems.

For detecting α-synuclein in tissue samples the membranes were fixed immediately after transfer with 4% paraformaldehyde (PFA), 0.1% glutaraldehyde in PBS for 30 minutes. Lack of fixation leads to poor/no α-synuclein detection and fixation with only 4% PFA also leads to lower detection than with the addition of 0.1% glutaraldehyde.

Following disposal of the fixation solution into properly designated containers for removal, the
membranes were blocked for 01:00:00 in 5% BSA in PBS Sigma Aldrich + 0.05% Tween-20 Sigma Aldrich Catalog #P9416 (PBS-T) at Room temperature.

7 The membranes were then incubated either overnight or for 01:00:00 at Room temperature in primary antibody probing for rat α-synuclein 1:1000 dilution α-Synuclein (D37A6) XP® Rabbit mAb Sigma Aldrich Catalog #4179.

8 The membranes were washed three times for 00:05:00 in PBS-T.

9 The membranes were incubated for 01:00:00 at Room temperature in secondary antibody 1:4000 Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab Sigma Aldrich Catalog #GENA934-1ML.

10 The membranes were washed five times for 00:05:00 in PBS-T.

11 The membranes were developed in SuperSignal® Trade; West Pico PLUS Chemiluminescent Substrate Sigma Aldrich Catalog #34579 and imaged in a

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The best signal for detection of alpha-synuclein from rat tissue was obtained on PVDF 0.45 μm with fixation in 4% PFA and 0.1% glutaraldehyde.

For recombinant human tau, fixation was not required and the membranes were instead immediately blocked for 1 hour in 5% BSA in PBS-T at Room temperature.

The membranes were then incubated either overnight or for 1 hour at Room temperature in primary antibody probing for human tau 1:200 dilution Polyclonal Rabbit Anti-Human Tau Unconjugated Ig fraction Sigma Aldrich Catalog #A002401-2.
15 The membranes were washed three times for 00:05:00 in PBS-T.

16 The membranes were incubated for 01:00:00 at Room temperature in secondary antibody, 1:4000 dilution

Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab Sigma Aldrich Catalog #GENA934-1ML

17 The membranes were washed five times for 00:05:00 in PBS-T.

18 The membranes were developed in Supersignal West Pico (#34580, ThermoFisher) and imaged in a

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The best signal for recombinant Tau was obtained on nitrocellulose 0.45 μm.

This optimisation was originally performed by Dr Colin Hockings, a former postdoc in the Molecular Neuroscience Group.