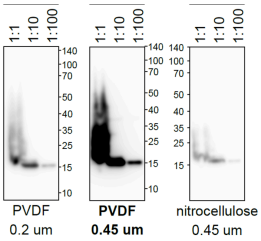
Jun 04, 2020

Selecting the correct transfer membrane for Western blot enhances detection of alpha-synuclein and tau proteins



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We use this protocol and it's working

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Abstract

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™ LDS Sample Buffer (4X) **Thermo Fisher Catalog #**NP0008
- NuPAGE™ 4-12% Bis-Tris Protein Gels, 1.5 mm, 15-well **Thermo Fisher Catalog #**NP0336PK2
- NuPAGE™ MES SDS Running Buffer (20X) **Thermo Fisher Catalog #**NP0002
- Immobilon-PSQ PVDF Membrane 0.2um roll **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**ISEQ00005
- Immobilon-P PVDF Membrane, 0.45um, roll **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**IPVH00010
- Nitrocellulose Transfer Membrane **Amersham plc Catalog #**10600002
- Methanol
- NuPAGE™ transfer buffer **Thermo Fisher Scientific Catalog #**NP0006
- PBS
- Tween-20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P9416
- Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab **Catalog #**GENA934-1ML
- SuperSignal™ 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 **Catalog #**GENA934-1ML

Protocol materials

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- α-Synuclein (D37A6) XP® Rabbit mAb **Cell Signaling Technology** **Catalog #**4179
- Nitrocellulose Transfer Membrane **Amersham plc** **Catalog #**10600002
- PBS
- Tween-20 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P9416

Safety warnings

Follow safety guidelines and wear correct PPE when handling fication solution containing 4% paraformalsehyde and 0.1% gluteraldehyde

1 Protein samples were boiled for 00:03:00 in 1x
NuPAGE™ LDS Sample Buffer (4X) **Thermo Fisher Catalog #**NP0008
and ran on
NuPAGE™ 4-12% Bis-Tris Protein Gels, 1.5 mm, 15-well **Thermo Fisher Catalog #**NP0336PK2
in
NuPAGE™ MES SDS Running Buffer (20X) **Thermo Fisher Catalog #**NP0002
using the

Equipment

XCell SureLock Mini-Cell Electrophoresis System	NAME
Electrophoresis System	TYPE
Invitrogen	BRAND
EI0001	SKU

at 200 V for 00:30:00 .

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

3 PVDF membranes were first activated with methanol by incubating for 00:03:00 .
Nitrocellulose membranes do not need to be activated first.

4 The gel was transferred onto each membrane using the

Equipment	
Invitrogen XCell II™ Blot Module	NAME
Western blot transfer module	TYPE
Invitrogen	BRAND
EI9051	SKU

with
NuPAGE™ transfer buffer **Thermo Fisher Scientific** **Catalog #**NP0006
+ 20%
Methanol
at 30 V for 01:30:00 . The blot module was kept On ice and surrounded by ice to keep cool and prevent protein damage.

5

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 serparate 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 00:30:00 . 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.

6 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
+ 0.05%
Tween-20 **Merck MilliporeSigma (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 **Cell Signaling Technology 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 **Catalog #**GENA934-1ML

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

11 The membranes were developed in

SuperSignal™ West Pico PLUS Chemiluminescent Substrate **Thermo Fisher Catalog #**34579

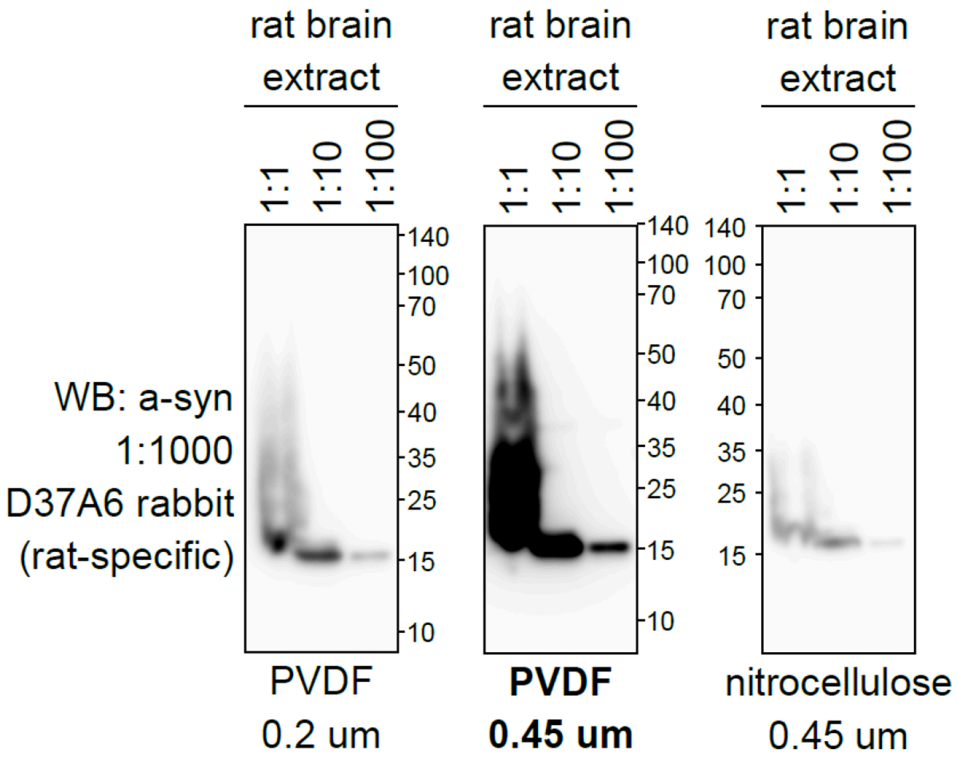
and imaged in a

Equipment	
G:Box Chemi	NAME
Imaging system	TYPE
Syngene	BRAND
XX6	SKU

.

12

Expected result



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.

13 For recombinant human tau, fixation was not required and the membranes were instead immediately blocked for 01:00:00 in 5% BSA in PBS-T at Room temperature .

14 The membranes were then incubated either overnight or for 01:00:00 at Room temperature in primary antibody probing for human tau 1:200 dilution Polyclonal Rabbit Anti-Human Tau Unconjugated Ig fraction **Agilent Technologies 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 **Catalog #**GENA934-1ML

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

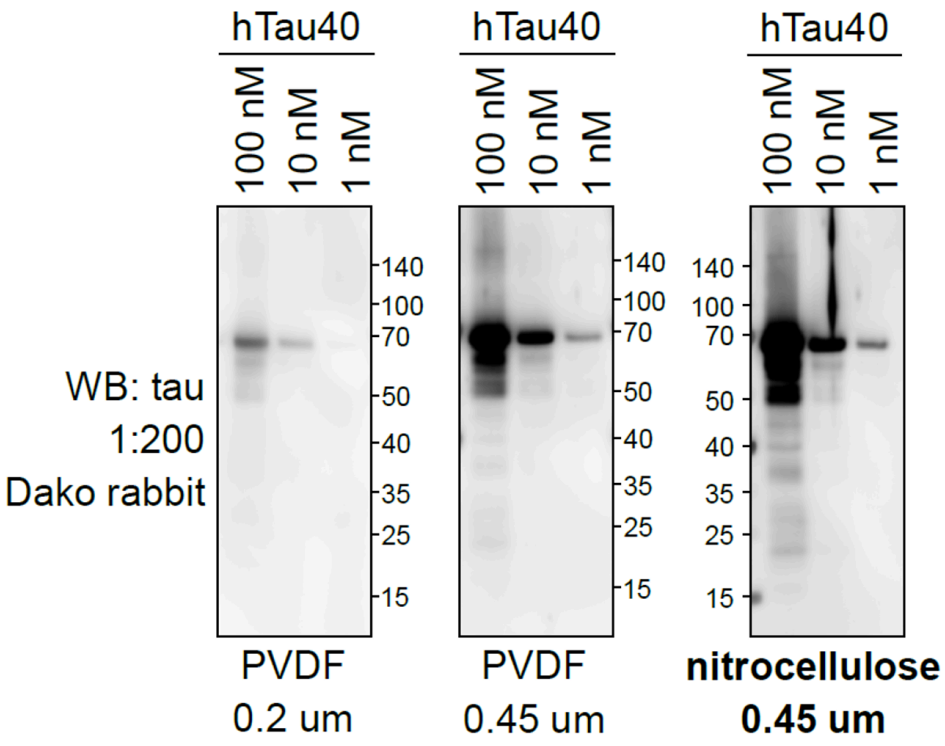
18 The membranes were developed in Supersignal West Pico (#34580, ThermoFisher) and imaged in a

Equipment	
G:Box Chemi	NAME
Imaging system	TYPE
Syngene	BRAND
XX6	SKU

.

19

Expected result



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

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