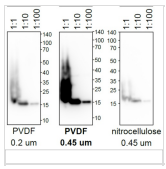

Jun 04, 2020

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

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1 Works for me dx.doi.org/10.17504/protocols.io.bg6kjzcw

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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.

DOI

dx.doi.org/10.17504/protocols.io.bg6kjzcw

PROTOCOL CITATION

Gabriele Kaminski Schierle 2020. Selecting the correct transfer membrane for Western blot enhances detection of alpha-synuclein and tau proteins. **protocols.io** dx.doi.org/10.17504/protocols.io.bg6kjzcw

KEYWORDS

Western blot, gel transfer, alpha-synuclein, tau, PVDF, nitrocellulose, fixation

CREATED

Jun 04, 2020

LAST MODIFIED

Jun 04, 2020

PROTOCOL INTEGER ID

37804

STEPS MATERIALS

NAME	CATALOG #	VENDOR
NuPAGE™ LDS Sample Buffer (4X)	NP0008	Thermo Fisher
NuPAGE™ 4-12% Bis-Tris Protein Gels, 1.5 mm, 15-well	NP0336PK2	Thermo Fisher
NuPAGE™ MES SDS Running Buffer (20X)	NP0002	Thermo Fisher
Immobilon-PSQ PVDF Membrane 0.2um roll	ISEQ00005	Millipore Sigma

NAME	CATALOG #	VENDOR
Immobilon-P PVDF Membrane, 0.45um, roll	IPVH00010	Millipore Sigma
Nitrocellulose Transfer Membrane	10600002	Amersham Biosciences
Methanol		
NuPAGE™ transfer buffer	NP0006	Thermo Fisher Scientific
PBS		
Tween-20	P9416	Sigma Aldrich
Rabbit IgG horse radish peroxidase (HRP) linked Whole Ab	GENA934-1ML	
SuperSignal™ West Pico PLUS Chemiluminescent Substrate	34579	Thermo Fisher
Polyclonal Rabbit Anti-Human Tau Unconjugated Ig fraction	A002401-2	Agilent Technologies
α-Synuclein (D37A6) XP® Rabbit mAb	4179	Cell Signaling Technology

EQUIPMENT

NAME	CATALOG #	VENDOR
G:Box Chemi	XX6	
XCell SureLock Mini-Cell Electrophoresis System	EI0001	Thermo Fisher Scientific
Invitrogen XCell II™ Blot Module	EI9051	Thermo Fisher Scientific

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)
by Thermo Fisher
Catalog #: NP0008

and ran on

NuPAGE™ 4-12% Bis-Tris Protein Gels, 1.5 mm, 15-well
by Thermo Fisher
Catalog #: NP0336PK2

in

NuPAGE™ MES SDS Running Buffer (20X)
by Thermo Fisher
Catalog #: NP0002

using the

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XCell SureLock Mini-Cell Electrophoresis System
Electrophoresis System
Invitrogen EI0001

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
by Millipore Sigma
Catalog #: ISEQ00005

PVDF 0.45 µm

Immobilon-P PVDF Membrane, 0.45um, roll
by Millipore Sigma
Catalog #: IPVH00010

and nitrocellulose 0.45 µm

Nitrocellulose Transfer Membrane
by Amersham Biosciences
Catalog #: 10600002

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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

Invitrogen XCell II™ Blot Module
Western blot transfer module
Invitrogen EI9051

with

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NuPAGE™ transfer buffer
by 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

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
by 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
by Cell Signaling Technology
Catalog #: 4179

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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
by Thermo Fisher
Catalog #: 34579

and imaged in a

G:Box Chemi
Imaging system
Syngene XX6

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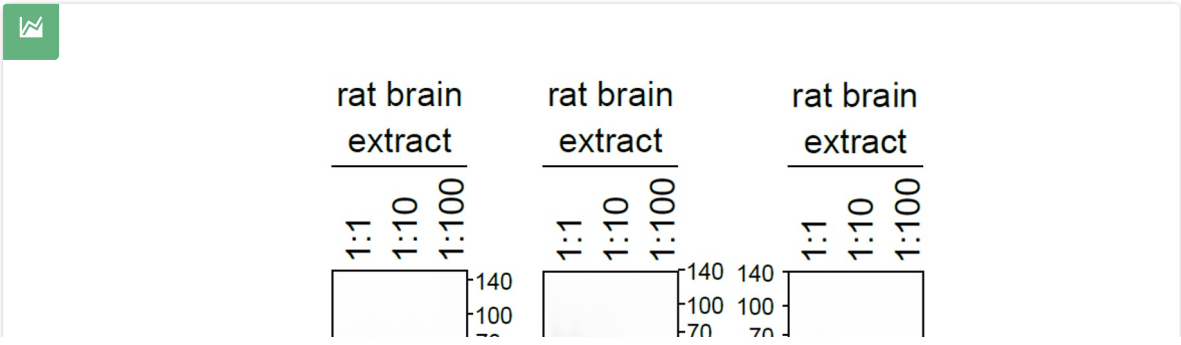
12



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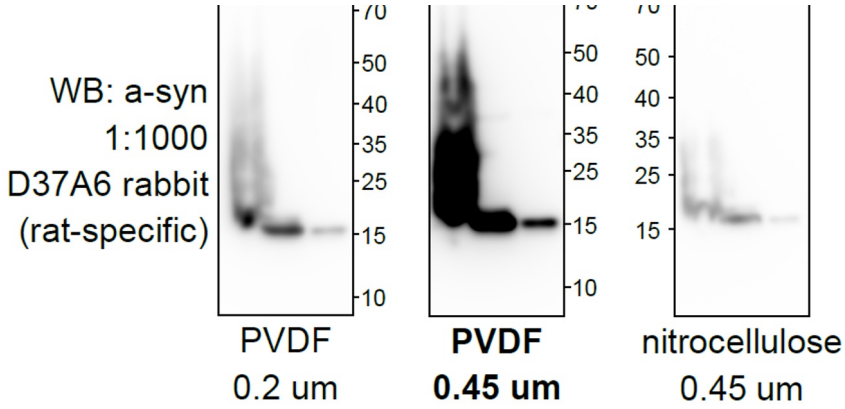

The best signal for detection of alpha-synuclein from rat tissue was obtained on PVDF 0.45 µm with fixation in 4% PFA and 0.1% glutaraldehyde.

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
by 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.

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

G:Box Chemi
Imaging system
Syngene XX6

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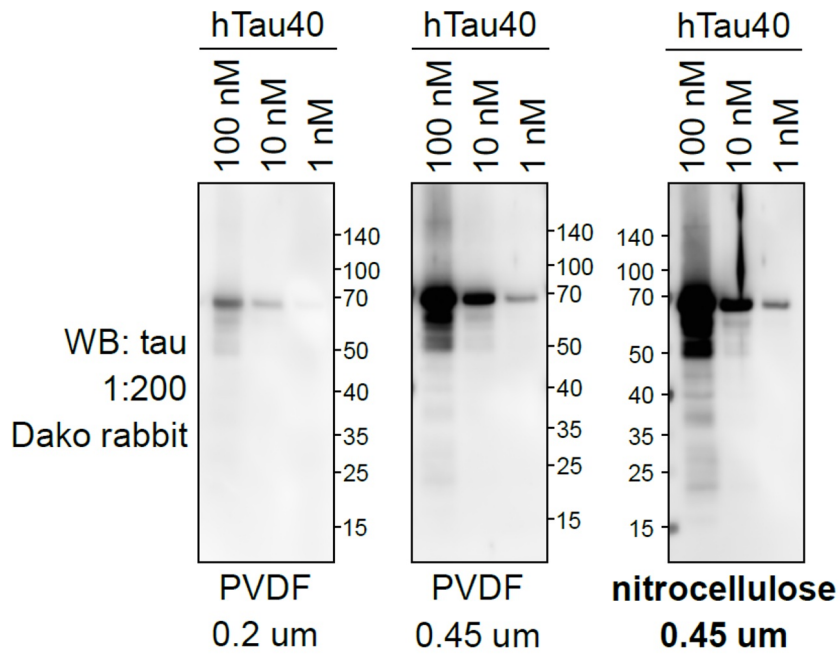
19



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.

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