



May 17, 2024

Western blot - alpha-synuclein



Forked from [Western blot - alpha-synuclein](#)

DOI

dx.doi.org/10.17504/protocols.io.rm7vzjb22lx1/v1

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Protocol Citation: Pietro La Vitola, Eva M. Szegö 2024. Western blot - alpha-synuclein. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.rm7vzjb22lx1/v1>

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Protocol status: Working

We use this protocol and it's working



Created: May 17, 2024

Last Modified: May 17, 2024

Protocol Integer ID: 99998

Keywords: alpha-synuclein, western blot, dosal medulla oblungata, synuclein protein, synuclein, synuclein this protocol, mouse dorsal medulla oblongata tissue, mouse dorsal medulla, mouse

Funders Acknowledgements:

Aligning Science Across Parkinson's

Grant ID: ASAP-000420

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Abstract

This protocol describes how to detect alpha-synuclein protein in mouse dorsal medulla oblongata tissue by western blot



Materials

Laemmli SDS sample buffer, not reducing

 Laemmli SDS-Sample buffer, not reducing Thermo Scientific Catalog #J60660.AC

Towbin transfer buffer

25 mM Tris

192 mM glycine

 8.3

20% methanol (vol/vol).

1 L of buffer:

- 800 mL distilled H₂O
- 200 mL methanol
- 3.03 g Tris base
- 14.4 g Glycine

Troubleshooting



Sample preparation

1d 4h 16m

1 *Tissue homogenization*

1.1 Homogenise samples in 150 μ L ice-cold lysis buffer (1% Triton X-100 in 0.1 M phosphate buffered saline solution, pH 7.6 , supplemented with protease and phosphatase inhibitors.

1.2 Centrifuge samples $\hookrightarrow$ 14000 x g, 4°C, 00:30:00

30m

1.3 Transfer supernatants into pre-cooled test tubes.

1.4 Measure protein concentration (e.g. BCA method).

1.5 Mix 4 μ g samples in Laemmli sample buffer supplemented with 5 % beta-mercaptoethanol.

1.6 Heat samples 🔥 95 °C 🕒 00:05:00

5m

2 *Electrophoresis and transfer*

2.1 Separate samples (4 μ g total protein) by polyacrylamide gel electrophoresis using precast Bolt™ 4-20% Bis-Tris, 1.0 mm, Mini Protein Gels at 120 V 🕒 01:20:00 or until dye front reaches the bottom of the gel. Run with pre-stained size markers

1h 20m

2.2 Soak nitrocellulose membrane (pore size: 0.2 μ m) with Towbin buffer (see materials)

2.3 Soak transfer sandwich components (2 sheets of filter paper and 2 blotting pads) in Towbin transfer buffer and assemble in the transfer cassette in the following order:
cathode plate
1 x blotting pad
1 x filter paper



gel
nitrocellulose membrane
1 x filter paper
1 x pad
Use a roller to remove any air bubbles

2.4 Place cassette in transfer tank and transfer protein onto 0.2 µm nitrocellulose membranes (300 mA,  01:30:00 in cold Towbin buffer. 1h 30m

2.5 Rinse membranes with TBS  00:00:30 30s

3 *Immunodetection of protein bands*

3.1 Block non-specific binding sites with blocking solution (TBS-T: TBS containing 2% BSA and 0.05 % Tween-20) for  01:00:00 1h

3.2 Incubate membranes in blocking solution containing mouse anti-α-synuclein (1:1000; RRID:AB_398108) and rabbit anti-β-actin (1:1000; RRID:AB_2305186)  18:00:00 18h
 Room temperature

3.3 Wash with TBS-T  00:05:00 4x 5m

3.4 Incubate membrane with peroxidase-conjugated goat anti-mouse and goat anti-rabbit IgG in TBS-T (1:5000 each).  00:00:00 RT

3.5 Wash with TBS-T  00:05:00 4x 5m

3.6 Incubate membrane with ECL  00:00:30 30s

3.7 Visualize ECL stained membrane using a BioRad ChemiDoc™.



Protocol references

Citation

Szegö EM, Van den Haute C, Höfs L, Baekelandt V, Van der Perren A, Falkenburger BH (2022)
. Rab7 reduces α -synuclein toxicity in rats and primary neurons..

<https://doi.org/10.1016/j.expneurol.2021.113900>

LINK

Citations

Szegö EM, Van den Haute C, Höfs L, Baekelandt V, Van der Perren A, Falkenburger BH. Rab7 reduces α -synuclein toxicity in rats and primary neurons.

<https://doi.org/10.1016/j.expneurol.2021.113900>