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Western blot - alpha-synuclein

Forked from Western blot - alpha-synuclein

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We use this protocol and it's working

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Disclaimer

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
pH 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 µL 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 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 µg 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 µg 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 µm) 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..

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Citations

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