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Protocol status: Working
We use this protocol and it's working

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Western blot for tissue extract

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ABSTRACT

Protocol for the detection of proteins by Western blot from tissue extract

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MATERIALS

Recipe for 100 mL 1x RIPA buffer :

- 5 mL Tris pH 7.4
- 1 mL Triton x100
- 1 g Deoxycholate
- 3 mL 5M NaCl
- 200 µL 0.5M EDTA (pH 8.0)
- 1 mL 10% SDS

Add water until 100 mL (MilliQ water)
Add protease and phosphatase inhibitors (Pi + PPi) right before use

Reagents used during Western blotting :

- 4X Laemmli buffer
- PageRuler™ Plus Prestained Protein Ladder
- 4–15% Criterion™ Tris-HCl Protein Gel
- Trans-Blot® TurboTM PVDF Membrane
- 5% milk powder dissolved in PBS-T 0.1% (PBS + 0.1% Triton-X100)
- Goat anti-rabbit HRP secondary antibody (Dako)
- ECL Prime chemiluminescence kit (GE Healthcare)

Recipe for 10.2 mL 4x Laemmli buffer (0.24M Tris pH 6.8, 7.27% SDS, 40% Glycerol, 10% b-Mercaptoetanol, 0.01% Bromophenol blue):

- 2.4 ml 1M Tris pH6.8
- 0.8g SDS
- 4ml 100% glycerol
- 2.8ml dH2O
- 1ml b-mercaptoethanol
- 0.01% Bromophenol blue

BEFORE START INSTRUCTIONS

Perform protein extraction from snap-frozen brain tissue:

- weigh tissue
- add RIPA buffer (see Materials) : 10 X of the weight (40 mg = 400 µL)
- homogenize samples using sample homogenizer
- sonicate samples at 4 degrees C, 3 times 15 seconds (keep the samples on ice between each sonication)
- centrifuge samples at 6000 g for 10 minutes at 4 degrees C
- collect supernatant and measure protein concentration
- aliquot protein extracts and store at -20 degrees

Day 1

1 Prepare sample for western blot 10m

15 µg of protein in 12 µL PBS

Add 4 µL Laemmli buffer

Boil at 98 °C for 00:10:00

2 Load samples, together with 7 µL of mass marker (PageRuler™ Plus Prestained Protein Ladder) on a on 4–15% Criterion™ Tris-HCl Protein Gel.

3 Run the gel for 00:10:00 at 80V 10m

4 Run the gel for 00:45:00 at 150V 45m

5 Transfer the proteins on PVDF membrane (Trans-Blot® TurboTM PVDF Membrane) using Trans-Blot Turl 30m Transfer System (Bio-Rad), using the pre-programmed protocol STANDARD SD: 00:30:00 , up to 1.0 A, 25 V.

6 Block membranes for 01:00:00 using 5% milk dissolved in PBS-T 0,1% at Room temperature 1h

7 Incubate membranes Overnight in primary antibody in 5% milk PBS-T 0,1% at 4 °C

Day 2

8 Wash membranes with PBS-T 0,1% for 10 minutes, 3 times at T Room temperature 30m

Wash PBS-T 00:10:00

Wash PBS-T 00:10:00

Wash PBS-T 00:10:00

9 Incubate membranes for 01:00:00 horseradish peroxidase-conjugated secondary antibody (Dako) diluted 1/10000 in 5% milk PBS-T 0,1% at Room temperature 1h

10 Wash membranes with PBS-T 0,1% for 10 minutes, 3 times at T Room temperature 30m

Wash PBS-T 00:10:00

Wash PBS-T 00:10:00

Wash PBS-T 00:10:00

11 Develop membranes using ECL Prime (GE Healthcare)