Jan 09, 2024 Version 2

Western blot in homogenised mouse brain samples V.2

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.yxmvm26m5g3p/v2

Katherine Brimblecombe[1,2,3], Natalie Connor-Robson[4], Stephanie J Cragg[1,2,3]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, OX1 3PT, UK;
[2]Oxford Parkinson's Disease Centre, University of Oxford, Oxford, United Kingdom;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815;
[4]Oxford Parkinson's Disease Centre, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK.

Cláudia C. Mendes
University of Oxford

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.yxmvm26m5g3p/v2

Protocol Citation: Katherine Brimblecombe, Natalie Connor-Robson, Stephanie J Cragg 2024. Western blot in homogenised mouse brain samples. **protocols.io https://dx.doi.org/10.17504/protocols.io.yxmvm26m5g3p/v2**Version created by **Cláudia C. Mendes**

Manuscript citation:
Brimblecombe KR, Connor-Robson N, Bataille CJR, Roberts BM, Gracie C, O'Connor B, Te Water Naude R, Karthik G, Russell AJ, Wade-Martins R, Cragg SJ. Inhibition of striatal dopamine release by the L-type calcium channel inhibitor isradipine co-varies with risk factors for Parkinson's. Eur J Neurosci. 2023 Nov 8. doi: 10.1111/ejn.16180. Epub ahead of print. PMID: 37941514.

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

Created: December 20, 2023

Last Modified: January 09, 2024

Protocol Integer ID: 92554

Keywords: western blot analysis of protein, homogenised mouse brain sample, homogenised mouse brain samples this protocol, western blot analysis, western blot, mouse brain, using novel antibody, antibody, novel antibody, assay

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-020370

Abstract

This protocol is for western blot analysis of proteins in homogenised mouse brain samples. The sensitivity and selectively of this assay is dependent on the efficacy of antibodies. When using novel antibodies ensure they have been validated for western blot, ideally using tissue from knock-out animals.

Guidelines

Due to the samples being homogenised, biological replicates should be from individual animals. Technical replicates run on repeated samples from the same animal can be illustrative of the reliability of each reading, however, they do not reflect biological variability.

Materials

Reagents:

- Tris (vendor, CAT#, URL)
- NaCl
- NP-40
- NaDeoxycholate
- SDS
- Protease Inhibitor cocktail tablet
- PhosSTOP tablet
- β-mercaptoethanol
- glycerol
- Bromophenol blue
- glycine
- MetOH
- Bovine Serum Albumin (BSA)
- chemiluminescent ECL Substrate Kit

Equipment:

- Tissue Tearor
- Spectrophotometer
- Electrophoresis machine

Preparing RIPA Buffer:

- 50 mM Tris pH8 0.606 g/100ml
- 150 mM NaCl 0.877 g/100ml
- 1 % NP-40
- 0.5 % NaDeoxycholate
- 0.1 % SDS

For each 10 mL add 1x Protease Inhibitor cocktail tablet and 1x PhosSTOP tablet.

Preparing Loading Buffer (6 X):

- 12 % SDS (1.2g / 10 ml)
- 30 % β-mercaptoethanol
- 60% glycerol
- 0.012 % Bromophenol blue
- 375 mM Tris pH 6.8

Preparing WB Running buffer (10X):

To make 1L:

- 144g glycine
- 30.3 g Tris base

- 10 g SDS
- MilliQ up to 1 L

Preparing TBS (10X):

To make 1L:

- 200 mL Tris 1 M pH 7.5
- 80g NaCl
- MilliQ up to 1L

To make TBST, add Triton at 1%.

Preparing Transfer buffer (10X):

To make 1L:

- 144g glycine
- 30.3 g Tris base

To dilute to 1X:

- 750 mL dH2O
- 150 mL MetOH
- 100 mL 10X Transfer buffer

Troubleshooting

Preparing Samples

1 Take samples out of the -80°C freezer and keep on dry ice until ready to digest.

2 On wet ice, add 200 µL RIPA Buffer (see **Materials**) to each unilateral striatum sample.

3 Mix thoroughly until sample completely blended with Tissue Tearor.

4 Centrifuge samples at 6g for 5 min at 4ºC.

5 Using the supernatant, dilute samples to about 1:10 to determine protein concentration.

Analysing Protein Concentration

6 **Prepare pre-diluted/standards:** 1.0, 0.8, 0.6, 0.4, 0.2 and 0.0 mg/mL of Bovine Serum Albumin (BSA)

7 **Prepare solution A:**
- 320 µL Copper (II) Sulfate Solution
- 16 mL Bicinchoninic Acid Solution

8 In a well plate, add 10 µL of pre-diluted samples/standards + 100 µL solution A.

Do this in triplicate.

9 Cover plates with film and incubate the samples for 30 min at 37ºC.

10 Measure the absorbance of each sample at 562 nm using a spectrophotometer and create a standard curve to determine concentrations.

Running Western Electrophoresis Gel

11 Make up 83 µL samples in RIPA Buffer at a concentration of 20 µg/10 µL and then add 17 µL of 6x Loading Buffer (see **Materials**).

12 Boil samples at this stage at 95 ºC for 5 min.

Note

Always check antibody data sheet before performing this step.

13 Prepare 1X Running Buffer (see **Materials**) and add the pre-cast gel cassettes (4 – 12%).

14 Load 5 µL of sample per well.

15 Load visual + developable ladder in first lane, and only developable ladder in last lane.

16 Run electrophoresis for 60 min @ 200V/100mA.

17 Transfer gel onto membrane and transfer using machine.

Running Antibodies

18 Wash membranes with TBST (see **Materials**) for approximately 1 min and repeat 4x.

19 Make up 50 mL TBST solution with 4% milk (dried powder).

20 Roll membranes into 50 mL conical with 3 mL of TBST w/ Milk and Primary Antibody.

The following primary antibody (working concentration 1:1000) was used in Brimblecombe, K. et al. (2023): anti-calb1 (Cell Signalling #13176).

Note

Primary Antibody Solutions can be re-used several times.

21 Incubate overnight at 4 ºC or ~2 hours at room temperature.

22 Wash membranes with TBST for approximately 1 min and repeat 4x.

23 Incubate membrane in TBST w/ Milk and Secondary Antibody HRP conjugated (1:3000) for 1 hour at room temperature.

Note

Secondary Antibody Solutions can be re-used several times.

24 Wash membranes with TBST for approximately 1 min and repeat 4x.

25 Develop membranes with the chemiluminescent ECL Substrate Kit (~ 1 mL of each component).

26 Visualize gel on a Gel Doc System.

27 Put membrane back into TBST w/ Milk and the 1º/2º β-actin antibody (already conjugated, 1:50000).

28 Repeat **steps 25 and 26**.