protocols.io    Part of SPRINGER NATURE

Aug 21, 2025

## 🌐 Western blot for tissue extract

📄 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.q26g79q31vwz/v1

María Sanchiz Calvo[1], Eduard Bentea[1], Veerle Baekelandt[1]

[1]KU Leuven. Neurobiology and Gene therapy lab

**M** **María Sanchiz Calvo**
KU Leuven

---

## Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

[Create free account]

---

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

---

# Abstract

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

## Materials

Recipe for 100 mL 1x RIPA buffer :

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

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

## Troubleshooting

# Before start

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® Turbo™ PVDF Membrane)          `30m`
using Trans-Blot Turbo 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         `1h`

🌡 Room temperature

**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)