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We use this protocol and it's working

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Immunoblot

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ABSTRACT

Immunoblot of rodent brain regions

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1 Put the frozen bulk dissections from a specific brain region in a tube with lysis buffer (50mM Tris HCl, pH 7.0; 150 mM NaCl; 5 mM EDTA; 1% SDS; NP40-1%) supplemented with protease inhibitors (Roche).

2 Homogenize the tissue with syringes, pestles or a tissue homogenizer with drill. Keep on ice during homogenization.

3 Clarify the sample by centrifugation at 13000 rpm for 30 min at 4ºC. Collect the supernatant into a new tube.

4 Determine the protein concentration using the BCA method.

5 Resolve proteins in a 10 or 15 % polyacrylamide gel and transfer onto 0.45 µm nitrocellulose membranes (Amersham).

6 Block the membrane with 5% milk powder in PBS1X.

7 Incubate the membrane with the primary antibodies diluted as needed overnight at 4ºC.

8 Incubate the membrane with the secondary antibodies goat anti-rat, donkey anti-rabbit and sheep anti-mouse (all 1:1000, from Amersham) 1h at RT.

9 Image protein bands using either West Pico SuperSignal Substrate or SuperSignal West Femto (Thermo Fisher Scientific, 34080 and 34095, respectively) on an ImageQuant RT ECL imaging system (GE Healthcare).

10 Determine band densitometry by using ImageJ image analysis software (RRID:SCR_003070, https://imagej.net/). Normalize the levels of each protein of interest to β-actin expression.