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Immunoblot

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Protocol status: Working

We use this protocol and it's working

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Abstract

Immunoblot of rodent brain regions

- 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 4°C. 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 4°C.
- 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.