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Immunoblotting Method

Samples

Protein extraction performed in a buffer consisting of: Tris 10 mM, EDTA1mM, proteases inhibitors pH 7.4 (e.g. Protease inhibitor Kit of Quartett, Germany or cOmplete of Roche-Sigma (cat. Nr. 04693159001 Roche).

The extraction of the proteins from the tissue is done in the above buffer by sonication (2 x 10 seconds on ice, followed by centrifugation at 14'000 rpm, during 10 min at 4°C).

Sample loading

30 to 50 ug proteins / lane

Controls: cerebellum or muscle (EDL: extensor digitorum longus)

SDS-gel: 12 or 15% SDS-polyacrylamide gel

Transfer of the protein

Semi-dry for 1h, with 60 mA / membrane.

Transfer buffer: Tris Base 25 mM,Glycine 192 mM (pH8.3) 200 ml methanol for 1 L.

Primary and secondary antibodies: primary antibody at a dilution of 1:2'000 to 1:10'000 in 2% milk/TBST, 4°C overnight.

Secondary antibodies (e.g. rabbit) Sigma A0545, diluted 1:10'000, 2h at room temperature, 2& milk/TBST

Detection system

Luminata forte (Millipore) or WesternBright Quantum of Advansta

Important for taking pictures with an automatic camera: if you have very strong bands in the upper molecular weight, hide them with a sheet of black paper and repeat the detection there were at first sight you had no band (e.g. 12 KDa).