Western Blot Analysis

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We use this protocol and it's working

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ABSTRACT

Western Blotting is a technique for the immunodetection of proteins using antibodies with fluorescent or chemiluminescent detection.

ATTACHMENTS

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MATERIALS

Materials

- 0.33 M sucrose
- 8 mM Hepes, pH 7.4
- Laemli SDS boiling buffer (Sigma)
- 9-12% SDS-polyacrilamide gel
- polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ)
- 5% non-fat dry milk
- TBST

Western Blot Analysis

4h 40m

1 Prepare protein extracts as previously described.

2 Homogenize tissues in lysis buffer ([M] 0.33 Molarity (M) sucrose/ [M] 8 millimolar (mM) Hepes, pH 7.4 and protease inhibitors) and quantify them using the BCA protein determination method (Bio-Rad, Hercules, CA).

3 Dilute protein samples to equivalent volumes containing 20 µg of protein and boil in an equal volume of Laemli SDS boiling buffer (Sigma) for 00:10:00. 10m

4 Load samples into a 9-12% SDS-polyacrilamide gel and separate it by electrophoresis for 03:00:00 at 100 V. 3h

5 Transfer the proteins to polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ) for 01:30:00 at 300 mA. 1h 30m

6 After blocking of nonspecific binding with 5% non-fat dry milk in TBST, probe the membranes with primary antibodies and process.

7 Perform densitometric analysis using ImageQuantity One.

8 Normalize data to β-actin, normalize values of phosphorylated GSK-3β ($pTyr^{216}$ GSK-3β); phosphorylated α-syn ($pSer^{129}$ α-syn) and phosphorylated tau ($pSer^{396}$ tau) to total GSK-3β, α-syn, and tau, respectively, before statistical analysis of variance and express values as percent changes (%) of WT controls.

9 Dashed lines (in white) indicate discontinuous bands (nonsequential lanes) taken from the same blot, at the same molecular weight (mass – kDa) in order to better represent the mean signal from all values (5-6 individual blots/genotype/treatment) for that particular group. Corresponding control bands (loading controls) match experimental bands.