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**ABSTRACT**

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

**PROTOCOL**

Protein extracts were prepared as previously described.Tissues were homogenized in lysis buffer (0.33 M sucrose/8 mM Hepes, pH 7.4 and protease inhibitors) and quantified using the BCA protein determination method (Bio-Rad, Hercules, CA). Protein samples were diluted to equivalent volumes containing 20 µg of protein and boiled in an equal volume of Laemli SDS boiling buffer (Sigma) for 10 min. Samples were loaded into a 9-12% SDS-polyacrilamide gel and separated by electrophoresis for 3 h at 100 V. Proteins were transferred to polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ) for 1.5 hr at 300 mA. After blocking of nonspecific binding with 5% non-fat dry milk in TBST, the membranes were probed with primary antibodies and processed. Densitometric analysis was performed using ImageQuantity One. Data were normalized to β-actin, values of phosphorylated GSK-3β (pTyr216 GSK-3β); phosphorylated α-syn (pSer129 α-syn) and phosphorylated tau (pSer396 tau) were normalized to total GSK-3β, α-syn, and tau, respectively, before statistical analysis of variance and values expressed as percent changes (%) of WT controls.

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.