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## Western Blot Analysis

Federico

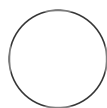
michela.deleidi<sup>1,2</sup>, Bianca Marchetti<sup>3,4</sup>, Bertoli<sup>1,2</sup>, Carmela Giachino<sup>3</sup>

<sup>1</sup>Mitochondria and Inflammation in Neurodegenerative Diseases, DZNE, Tübingen-Germany;

<sup>2</sup>Hertie Institute for Clinical Brain Research, University of Tübingen;

<sup>3</sup>Neuropharmacology Laboratory, Oasi Research Institute-IRCCS, Troina, Italy;

<sup>4</sup>Biomedical and Biotechnological Sciences, Pharmacology Section, University of Catania-Italy



carmela.giachino

### ABSTRACT

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

### ATTACHMENTS

[k4dwb48if.docx](#)







### 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,  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  $\mu\text{g}$  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  $\beta$ -actin, normalize values of phosphorylated GSK-3 $\beta$  (pTyr<sup>216</sup> GSK-3 $\beta$ ); phosphorylated  $\alpha$ -syn (pSer<sup>129</sup>  $\alpha$ -syn) and phosphorylated tau (pSer<sup>396</sup> tau) to total GSK-3 $\beta$ ,  $\alpha$ -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.