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Total and PS129 aSyn levels using Western Blot

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ASAP Collaborative Rese… Kaplitt Protocols

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We use this protocol and it's working

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Abstract

Measure total and ps129 alpha-synuclein in mouse plasma.

1 Dilute plasma samples 10-fold in MilliQ water.

2 Mix the sample in a 1:1 ratio with 20 mM TCEP to reduce disulfide bridges, and then 1:1 with a denaturing loading buffer (50 mM Tris-HCl, 70 mM Tris, 1% lithium dodecyl sulphate (LDS), 5% glycerol, 0.25mM EDTA, 0.11mM SERVA Blue G-250, 0.0875mM Phenol Red, pH-8.5). Boil at 95°C for 5 min.

3 Separate 20 µL of each sample using a 4-12% bis-tris acrylamide gel using MES running buffer (50mM Tris, 50mM 2-(N-morpholino)ethanesulfonic acid (MES), 0.1% SDS, 1mM EDTA, pH-7.3).

4 After Sodium dodecyl sulphate polyacrylamide gel electrophoresis, incubate gels in a transfer buffer (25mM Tris, 192mM glycine, 30% methanol) and 0.45µm pore size Immobilon-P™ PVDF membranes
(Millipore, MA, US). Pre-wet membranes in methanol.

5 Assemble gels with membranes. Perform transfer using the Trans-Blot Turbo Transfer System (BioRad, CA, US) according to manufacturer protocols.

6 Dry membranes and fix quickly in 100% methanol.

7 Between each incubation period, membranes were
washed three times in Tris Buffered Saline (TBS, 20mM Tris, 150mM NaCl, pH-7.6) containing 0.1%(v/v) Tween20 (TBS-T).

8 Block membranes for 01:00:00 in 5% skim milk at Room temperature . 1h

9 Incubate membranes Overnight at 4 °C with a primary antibody diluted 1:1000(v/v) in 1% skim milk. 1h

Antibodies used were:
-Anti-Total-Alpha-Synuclein Polyclonal Antibody (Thermo Fisher Scientific, PA5-143581, MA, US)
-Anti-Alpha-synuclein (phospho S129) antibody [EP1536Y] (Abcam, ab51253, UK)

10 Incubate membranes for 02:00:00 with appropriate HRP-conjugated secondary antibody (Dako/Agilent, CA, US) diluted 1:10000(v/v) in 1% skim milk at RT. 2h

11 Develop the signal with Clarity™ Western ECL Substrate (Bio-Rad, CA, US). Visualize levels of proteins by measuring band intensities with ImageJ.

12 Normalized target protein amounts to total protein in the sample, measured by BCA assay according to manufacturer instructions (Thermo Fisher Scientific, MA, US).

13 Use fluorescent scan to visualize the protein ladder.

Protocol references

Paslawski W, Svenningsson P. Elevated ApoE, ApoJ and lipoprotein-bound α-synuclein levels in cerebrospinal fluid from Parkinson's disease patients - Validation in the BioFIND cohort. Parkinsonism Relat Disord. 2023 Nov;116:105765. doi: 10.1016/j.parkreldis.2023.105765. Epub 2023 Jul 12. PMID: 37479568.