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Total and PS129 aSyn levels using Western Blot

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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-PTM 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.