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Western Blot

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We use this protocol and it's working

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Abstract

Western Blot Protocol

Troubleshooting

Cell lysis preparation

50m

1 Prepare the appropriate amount of RIPA buffer with Protease and Phosphatases Inhibitor Cocktail (Roche) and maintain On ice

2 Collect cells/organoids in PBS On ice in an eppendorf and spin down 500 rpm, 4°C, 00:05:00 5m

3 Add appopriate amount of lysis buffers (1:50 v/v proportion between cell pellet and lysis buffer) to the pellet and pipette up and down

3.1 For organoid samples use an homogeniser

4 Incubate samples for 00:30:00 On ice . Spinning every 00:10:00 40m

5 Centrifuge at 15000 x g for 00:10:00 at 4 °C and collect the supernatant in a new tube. 10m

6 If SDS fraction is desired, add [M] 2 Mass / % volume SDS to RIPA buffer and resuspend pellet from step 5.

6.1 Sonicate the sample a 3 times for 00:00:10 to ensure fragmentation of genomic DNA released during lysis and consequently reduce viscosity 10s

7 Quantify protein concentration.

SDS-PAGE

8 Mix 20-30 µg of protein with Laemmli's loading buffer

9 Denaturate the proteins by incubation at 95 °C for 00:05:00 , spin briefly before loading 5m

10 Load pre-cast gel into Western Bloat apparatus and fill with Running Buffer (BioRad).

11 Load samples and protein ladder into gels, Run the gel at 120V for 00:45:00 to ensure protein separation. 45m

Protein Immunodetection

12 Transfer proteins into a PVDF membrane (previously activated with methanol and hydrated in ddH2O)

13 Remove membrane from transfer and place into a box with blocking buffer: 5% BSA In TBS-T (20mM Tris-HCl, 150mM NaCL pH8, 0.1% Tween20). Block for 01:00:00 1h

13.1 If alpha-synuclein is to be blotted, fix the membrane with 4% PFA in PBS for 00:30:00 prior to blocking 30m

14 Once blocked, sequentially probe the membrane for antibody staining and detection. Antibody dilution might need optimization.

14.1 Prepare primary antibody in 5% BSA in TBS-T and left incubating Overnight at 4 °C 30m

14.2 Wash membranes 3x 00:10:00 in TBS-T and then incubated for 01:00:00 at Room temperature with the appropriate HPR-tagged secondary antibody. 1h 10m

14.3 Membrane was washed 3x 00:10:00 in TBS-T 10m

15 Mix EZ-ECL solution 1:1 (v/v) and incubate on the membrane for 00:01:00 1m

16 Image membranes using a Licor Odyssey Chemioluminescence Imager.