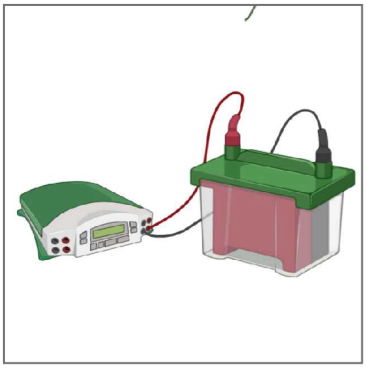

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Immunoblotting of α-synuclein species

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ABSTRACT

Immunoblotting (Western blotting) protocol is specifically designed to provide an information of protein separation, gel electrophoresis, transfer procedures optimized for a-synuclein protein.

PROTOCOL MATERIALS

Nitrocellulose Membrane, Roll, 0.2 um **Bio-Rad Laboratories Catalog #1620112**

Step 1.5

4–20% gradient mini-PROTEAN TGX stain-free gel **Bio-Rad Laboratories Catalog #4568085**

Step 1

PROTOCOL integer ID: 93930

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SDS-PAGE

1 Prepare stain-free gels in advance

4–20% gradient mini-PROTEAN TGX stain-free gel **Bio-Rad Laboratories Catalog #4568085**

1.1 Prepare buffers:

- 1x running buffer,
- 1x TBST,
- Transfer buffer (let cool in 4C while gel runs!)—Need around 1L for 2 gels

200ml meOH 100ml 10x transfer buffer 700ml ddH2O

1.2 Before starting

a. Prepare samples, loading order, and antibodies/concentrations [save to excel sheet]

1.3 Prepare gel

- Use 4-20% gel with 10/12/15 well comb (cold room)
- Open package, remove sticker from bottom
- Insert gel into cassette and close sides (if only running one gel add additional side filler)
- Fill inside of cassette with running buffer—make sure no leaks!
- Gently pull out comb

1.4 Loading samples

- Loading Samples:
- Outline loading order and save to data drive
- Load protein ladder (5ul) for each gel
- Optional: add 100pg recomb. synuclein

(1ul of stock 100pg/ul a-synuclein + Laemmli buffer in 10% DTT (fresh))

- Add 10ul to well
- Load 10ul to each well
- If not first time, calculate amount of sample if previously run (based off of synuclein total load)

- Vortex sample tube very well before loading
- Pipette slowly ensuring correct amounts are loaded
- Avoid the gunk

1.5 Run Gel and Set Up Transfer

- 90V for ~45min-1hr
- Once loading dye reaches the bottom of the gel stop it
- Soak the 0.2 um nitrocellulose membrane

  Nitrocellulose Membrane, Roll, 0.2 um **Bio-Rad Laboratories Catalog #1620112** in MeOH for at least 2 min
- Dump out the running buffer in the sink rinse the tank and cassettes with ddH2O
- Fill tank up half way with transfer buffer to prevent drying out
- Move the membrane to transfer buffer once ready to put together
- *Order of stacking:*

Black side of cassette down
Soak sponges with transfer buffer and place at bottom edge of black cassette
Soak one filter paper in transfer buffer and lay on sponge
Open gel carefully using wet spatula and cut the bottom and wells off gel
Orient the top of the gel (well 1 on top left) at the 'bottom' of the cassette.
Lay the nitrocellulose membrane on top of the gel make sure flat, avoid bubbles
Add another filter paper on top of nitrocellulose membrane
Put sponge on top and roll out the bubbles gently
Sandwich cassette closed and place in tank with Transfer buffer full

Check orientation of the cassette is black-black, red-red
Add ice pack to each tank (that is why only one cassette per tank)
Fill Transfer buffer to the top of the cassettes to make sure the membrane does not dry out.
Run overnight at 4C deli fridge, 30V

1.6 Stop Transfer, Block and Add Primary Antibody

1.7 Prepare blocking buffer (5% milk in 1x TBST)—always block in milk

1.8 Remove tank from 4C and dump out transfer buffer

1.9 Prepare tray for membranes (know which regions of the gel needed to cut) and label box

1.10 Remove cassettes and take off the sponge and filter paper

1.11 Prepare a container with TBST and use forceps to move the membrane from the gel to the TBST (make sure submerged)

1.12 Cut the membrane in the desired location based on the protein ladder and put in tray with TBST covering the membrane

2 After washes with TBST, add 5ml of blocking buffer to each membrane section

2.1 Block at room temp while rotating for 30minutes to 1 hour

In the meantime prepare/calculate antibody concentrations (update info on WB excel sheet)

2.2 After blocking, add antibody directly to corresponding membrane and put at 4C overnight

2.3 Place at 4C on shaker overnight

2.4 Wash 3x 5min TBST

2.5 **Wash and Add Secondary**

2.6 Dump out primary antibody solution

2.7 Wash blots 3x 5 min with TBST, with gentle rotation at room temp

2.8 Make fresh milk (5% in TBST) for secondary

2.9 Add secondary to blot, place in 4C rocking overnight

2.10 **Image Blot on BioRad Chemidoc**

2.11 **Quantify Blot**

a. Crop & Autoscale

b. Lane & Band:

i. Lane: manual, input number of lanes

ii. Adjust lanes

c. Bands:

i. Detect bands

d. Quantity tool

i. Select relative (WT)

ii. Click analysis table for all relative band values

e. Export table