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SDS and Clear Native Gel Electrophoresis

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We use this protocol and it's working

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Abstract

This protocol describes SDS–PAGE and clear native PAGE procedures for analyzing α-synuclein, Gba, and related proteins from mouse brain samples.

Materials

Materials and Reagents

- **Gels**
  4–20% precast SDS–polyacrylamide gels (Bio-Rad, 5671095)
  4–16% Bis-Tris Native Gels (Thermo, BN1002)

- **Sample Buffers**
  NativePage Sample Buffer (Thermo, BN2003)

- **SDS-PAGE Running Buffer**

25 mM Tris, 192 mM Glycine, 0.1% (w/v) SDS

- **Transfer Materials**
  Nitrocellulose membranes, 0.22 µm (1620115, Bio-Rad)
  Transfer buffer 1 L

	A	B
	Transferbuffer 10×	100 mL
	Methanol	200 mL
	DW	700 mL

- **CBB staining solution**
  Coomassie brilliant blue (Bio-Rad, 161-0406)

- **Standards**
  SDS-PAGE: Precision Plus Protein Standards (Bio-Rad, 161-0363)
  Native-PAGE: NativeMark protein standard (Invitrogen, LC0725)

- **Blocking and Wash Buffers**
  5% nonfat dry milk in TBS-T (0.05% Tween-20)

- **Secondary antibodies**
  IRDye 680LT or 800CW (LI-COR Biosciences)
  HRP-conjugated secondaries (for ECL detection)

- **Other Reagents**
  4% paraformaldehyde (PFA)

- **ECL-based Detection**
  ECL Prime Western Blotting Detection Reagents (Cytiva, RPN2232)
  High Performance chemiluminescence film (Cytiva, 28906836)

Troubleshooting

SDS–PAGE

1 Prepare protein samples and load them onto 4–20% precast SDS–polyacrylamide gels (Bio-Rad, 5671095).

2 Run electrophoresis following standard SDS–PAGE procedures.
Start at 80 V for approximately 30 minutes until the stacking is complete, then increase the voltage to 90–110 V to continue the separation.

3 Transfer proteins to 0.22 µm nitrocellulose membranes at 100 V for 1 h at 4°C.

4 (Optional for α-synuclein monomer detection)
Pretreat membranes with 4% PFA for 30 min before blocking.

5 Block membranes in 5% nonfat dry milk/TBS-T (0.05% Tween-20).

6 Incubate membranes with primary antibodies in 5% nonfat dry milk/TBS-T overnight at 4°C with gentle agitation.

7 Wash 3× 10 min with TBS-T.

8 Incubate with IRDye secondary antibodies for 1 h at room temperature.

9 Wash 3× 10 min with TBS-T.

10 Visualize and quantify signals using the Odyssey infrared imaging system.

Clear Native PAGE

11 Prepare samples in NativePage Sample Buffer.

12 Load samples onto 4–16% Bis-Tris native gels.

13 Run native PAGE at a constant 150 V for 2 h.
- Cathode buffer: 50 mM Tricine, 15 mM Bis-Tris, pH 7.0
- Anode buffer: 50 mM Bis-Tris, pH 7.0

Include NativeMark as the molecular weight standard.

14 Transfer proteins to 0.22 µm nitrocellulose membranes at 100 V for 1 h at 4°C.

15 Visualize standards on the gel using CBB staining.

16 Proceed with immunoblotting:
- Block membranes in 5% milk/TBS-T
- Incubate with primary antibodies overnight at 4°C
- Wash and incubate with IRDye-conjugated secondary antibodies for 1 h at room temperature
- Detect signal using the Odyssey imaging system

ECL-based Detection of Endogenous Gba

17 Incubate the membrane with primary antibody (diluted in 5% milk/TBS-T) overnight at 4°C with gentle agitation.

18 Wash 3× 10 min with TBS-T.

19 Incubate with HRP-conjugated secondary antibodies (diluted in 5% milk/TBS-T) for 1 h at room temperature with gentle agitation.

20 Wash 3× 10 min with TBS-T.

21 Incubate the membrane with Western Blotting Detection Reagents for 5 min before detection.

22 Expose onto chemiluminescence film or use a digital imaging system for signal detection.