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Western blotting to detect ATP13A2

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We use this protocol and it's working

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Abstract

Protocol to detect ATP13A2 via Western Blotting.

Materials

■ **Antibodies:**

- Goat anti-mouse IgG (H+L) secondary antibody HRP conjugated: Thermo Scientific, 31430
- Goat anti-rabbit IgG (H+L) secondary antibody HRP conjugated: Thermo Scientific, 31460
- Mouse monoclonal anti-GAPDH antibody (lot #067M4785V, dilution 1:5,000): Sigma, G8795.
- Rabbit anti-ATP13A2 antibody (lot #0000102992, dilution 1:1,000): Sigma, A3361.

■ TrypLE: Thermo Scientific, 11528856

■ Dulbecco's Phosphate Buffered saline modified without calcium chloride and magnesium chloride (DPBS): Gibco, D8537

■ Micro-BCA Protein Assay Kit: Pierce BCA Protein Assay Kit, Thermo Scientific, 23225

■ NuPAGE LDS sample buffer: Invitrogen, NP0007

■ Pre-cast 4-12% Bis-Tris gels: Invitrogen, NP0321BOX

■ PVDF membranes: Thermo Scientific, 88518

■ RIPA Lysis and Extraction Buffer: Invitrogen: 89900

■ SIGMAFAST Protease Inhibitor Cocktail Tablets, EDTA-Free: Sigma: S8830

■ SuperSignal West Pico PLUS chemiluminescent Substrate: Thermo Scientific, 34095

■ Transfer buffer: Life Technologies, NP0006-1

■ MES buffer: Thermo Scientific, NP0002



Harvesting cells

- 1 Collect the cells by using TrypLE.
- 2 Centrifuge cell suspensions at 450 xg, 4°C for 00:05:00 . 5m
- 3 Resuspend cell pellets with DPBS and centrifuge following the same indications as in **2**. Repeat once.
- 4 Discard supernatants and keep cell pellets on ice.

Cell lysis and protein concentration determination

- 5 Resuspend cell pellets in RIPA buffer (RIPA Lysis and Extraction Buffer) supplemented with protease cocktail inhibitors.
- 6 Vortex 00:00:30 and keep on ice for 00:10:00 . 10m 30s
- 7 Centrifuge at 20,000g, 4 °C for 00:10:00 . 10m
- 8 Keep supernatants on ice to proceed with protein concentration determination using the micro-BCA Protein Assay Kit.

SDS-PAGE and Western blot

7h 10m

- 9 **Loading**
- 9.1 Mix 10 µg of protein with NuPAGE LDS sample buffer and 5% β-mercaptoethanol final.
- 9.2 Boil for 00:05:00 at 95 °C 5m



9.3 Load protein on pre-cast 4-12% Bis-Tris gels. Include at least one lane with a protein ladder.

10 Running

10.1 Run for and  01:20:00 at 130V in MES buffer.

1h 20m

11 Transfer

11.1 Transfer onto PVDF membranes using a liquid transfer and following settings: 100V,  01:30:00, 4°C. Use transfer buffer with 10% methanol.

1h 30m

12 Blocking

12.1  01:00:00 Block membranes with blocking buffer (5% milk powder in 1X TBS and 0.1% Tween20 (REF)) for  01:00:00 at room temperature, 19 rpm.

2h

13 Primary antibodies

13.1 Incubate membrane with primary antibodies in solution (1% bovine serum albumin in 1X TBS-Tween20 (TBS-T) buffer),  Overnight at 4°C, 19 rpm.

1h

13.2 Wash membrane three times for  00:05:00 in TBS-T, 19 rpm.

5m

14 Secondary antibodies

14.1 Incubate membrane with peroxidase-conjugated secondary antibodies in solution (1% milk powder in 1X TBS-T) for  01:00:00 at room temperature and, 19 rpm.

1h



14.2 Wash membrane five times for  00:05:00 in TBS-T, 19 rpm.

5m

15 **Detection**

16 Use a chemiluminescence reagent to detect signal and acquire with a Biorad Camera (Vilber Lourmat) and its software (ImageLab).