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We use this protocol and it's working

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SDS-PAGE and western blot analysis

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ABSTRACT

This protocol describes SDS-PAGE and western blot analysis.

ATTACHMENTS

758-1926.pdf

MATERIALS

Materials

- 100 mM DTT
- PageRuler Prestained protein marker (Thermo Fisher)
- nitrocellulose membranes (RPN132D, GE Healthcare)
- Mini Trans-Blot Cell (Bio-Rad)
- 0.1% Tween 20
- horseradish peroxidase (HRP)-coupled antibodies

RIPA buffer

	A	B
	Tris-HCl pH 8.0	50 mM
	NaCl	150 mM
	sodium deoxycholate	0.50%
	SDS	0.10%
	NP-40	1%

cOmplete™, Mini, EDTA-free (Protease Inhibitor) **Roche Catalog ##11836170001)**

Roche PhosSTOP™ **Merck MilliporeSigma (Sigma-Aldrich) Catalog #4906837001**

Pierce™ Detergent Compatible Bradford Assay Kit **Thermo Fisher Catalog #23246**

SuperSignal™ West Femto Maximum Sensitivity Substrate **Thermo Fisher Catalog #34096**

SDS-PAGE and western blot analysis

10m

1 For SDS-PAGE and western blot analysis, collect cells by trypsinization and subsequent centrifugation at 300 x g, 4°C, 00:05:00. 5m

2 Wash the cell pellets in PBS and centrifuge once more at 300 x g, 4°C, 00:05:00. 5m

3 Remove the supernatant and lyse the cell pellets in RIPA buffer supplemented by cOmplete EDTA-free protease inhibitors (11836170001, Roche) and phosphatase inhibitors (Phospho-STOP, 4906837001, Roche).

4 After incubating in RIPA buffer for 00:20:00 On ice, clear the samples by centrifugation at 20000 x g, 4°C, 00:10:00. 30m

5 Collect the soluble supernatant fraction and measure the protein concentrations using the Pierce Detergent Compatible Bradford Assay Kit (23246, Thermo Fisher).

6 Then, adjust the samples for equal loading and mix it with 6x protein loading dye, supplemented with 100 mM DTT. Then, boil it for 00:05:00 at 95 °C. 5m

7 Load the samples on 4-12% SDS-PAGE gels (NP0321BOX, NP0322BOX, or NP0323BOX, Thermo Fisher) with PageRuler Prestained protein marker (Thermo Fisher).

8 Transfer the proteins onto nitrocellulose membranes (RPN132D, GE Healthcare) for 01:00:00 at 4 °C using the Mini Trans-Blot Cell (Bio-Rad). 1h

9 After the transfer, block the membranes with 5% milk powder dissolved in PBS-Tween (0.1% Tween 20) for 01:00:00 at Room temperature. 1h

10 Incubate the membranes Overnight at 4 °C with primary antibodies dissolved in the blocking buffer and wash. 1h

10.1 Wash for 00:05:00 .(1/3) 5m

10.2 Wash for 00:05:00 .(2/3) 5m

10.3 Wash for 00:05:00 .(3/3) 5m

10.4 Then, incubate with species-matched secondary horseradish peroxidase (HRP)-coupled antibodies diluted 1:10,000 in blocking buffer for 01:00:00 at Room temperature . 1h

11 Afterwards wash the membranes three times with PBS-T and further process for western blot detection.

12 Incubate the membranes with SuperSignal West Femto Maximum Sensitivity Substrate (34096, Thermo Fisher) and image it with a ChemiDoc MP system (Bio-Rad).

13 Analyze the images with ImageJ [57].