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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

OPEN ACCESS



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Protocol status: Working We use this protocol and it's working

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RIPA buffer

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

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

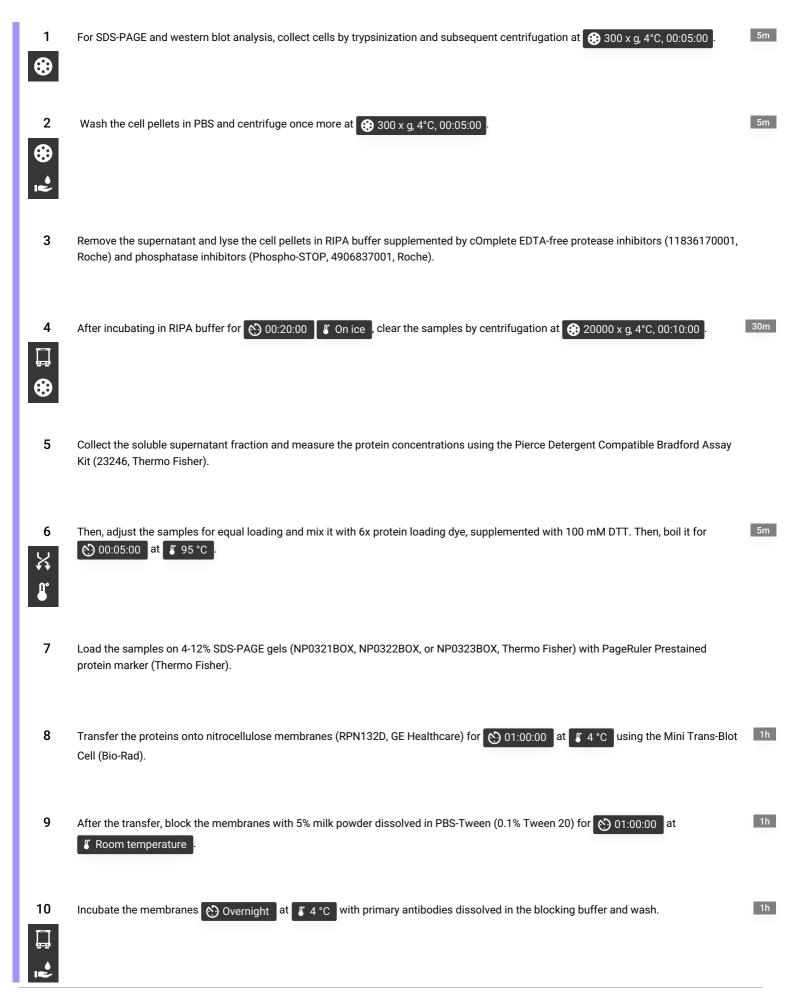
Roche PhosSTOP™ Merck MilliporeSigma (Sigma-Aldrich) Catalog #4906837001

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





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

5m

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

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 8 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).

Analyze the images with ImageJ [57].



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