1. Sample_prep_WB

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[1]In-house protocol

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

Sample preparation for Western bolt assay

PROTOCOL CITATION

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ABSTRACT

Sample preparation for Western bolt assay

BEFORE STARTING

NOTE: We had issues with the dye front showing up in the image, therefore we do not add Coomassie blue and Phenol red in our sample buffer.

To prepare 10 ml of 2X Tricine SDS Sample Buffer, mix the following reagents:

- 3 M Tris HCl, pH 8.45 3 ml
- Glycerol 2.4 ml
- SDS 0.8 g

- 0.1% Coomassie Blue G 0.5 ml
- 0.1% Phenol Red 0.5 ml

Mix well and adjust the volume to 10 ml with ultrapure water.
Store at 4 ° C. The buffer is stable for 6 months when stored at4 ° C.

Sample preparation for Western bolt assay

1 Grow cells, harvest, wash with DI water, and store cell pellet at -800C

2 Lysis buffer(50 mM Tris HCl pH 7.5, 200 mM NaCl, 5% Glycerol)- we don't add DTT or PMSF (https://www.embl.de/pepcore/pepcore_services/protein_purification/extraction_clarification/cell_lysates_ecoli/index.html)

For 100 ml

a. 1M TrisHClpH 7.55 ml
b. 5MNaCl4 ml
c. 80% Glycerol6.25 ml
d. Distilled waterupto100 ml

- Before use, add 1 tablet of a Protease inhibitor to 10 ml lysis buffer.
- After mixing with cells, add 300ug/ml final concentration lysozyme and let it sit on ice for 1-4 hrs.

3 Bead beating and separation of Cytoplasmic and Membrane fraction
a. Follow the"Bead Beating" protocol
b. Centrifuge high speed for 30 minutes to get supernatant (cytoplasmic) and pellet (membrane fraction)
If required to separate inner and outer membrane follow "Subcellular fractionation protocol".
If required perform acetone precipitation of the protein following "Acetone precipitation protocol".

4 Use the"Bradford assay" protocol to measure protein concentration and mix your protein sample with 2X Tricine sample buffer such that you have 0.5-1ug/ul of protein.

5 2X Tricine Sample buffer(Novex, 2003)

- 450mMTrisHCl
- 12% Glycerol
- 4% SDS
- 0.0025% Coomassie Blue G
- 0.0025% Phenol Red
- pH 8.45