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# 1. Sample\_prep\_WB

Elizabeth Fozo<sup>1</sup><sup>1</sup>In-house protocol

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Works for me

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## ABSTRACT

Sample preparation for Western bolt assay

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## KEYWORDS

Western Bolt assay, Western Bolt, Assay

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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 at 4 ° C.

#### Sample preparation for Western blot assay

- 1 Grow cells, harvest, wash with DI water, and store cell pellet at -80°C
- 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](https://www.embl.de/pepcore/pepcore_services/protein_purification/extraction_clarification/cell_lysates_ecoli/index.html))  
For 100 ml
  - a. 1M TrisHCl pH 7.5 55 ml
  - b. 5M NaCl 4 ml
  - c. 80% Glycerol 6.25 ml
  - d. Distilled water up to 100 ml
  - Before use, add 1 tablet of a Protease inhibitor to 10 ml lysis buffer.
  - After mixing with cells, add 300 µg/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 0.5-1 µg/µl of protein.
- 5 2X Tricine Sample buffer(Novex, 2003)
  - 450 mM Tris HCl
  - 12% Glycerol
  - 4% SDS
  - 0.0025% Coomassie Blue G
  - 0.0025% Phenol Red
  - pH 8.45