PROTOCOL

Proteomics Protocols for Focused-Ultrasonicators

Native Protein Extraction Using Covaris Adaptive Focused Acoustics (AFA)

Introduction

Covaris Adaptive focused acoustics (AFA) in conjunction with compatible lysis buffers can be used to disrupt yeast cells and release proteins for further analysis. AFA is a highly controllable acoustic based non-contact and isothermal process that preserves native protein conformation and biological activity in the presence of non-denaturing buffers.

This protocol describes the extraction of native proteins using the **Protein Extraction Buffer Super B (Kit PN 520112).** This buffer provides a non-denaturing environment for protein extraction using a non-detergent sulfobetaine (NDSB). NDSB has been shown to prevent protein aggregation [1], facilitates protein refolding [2], enhances protein antigenicity [3] and does not form micelles. NDSB is easily removed from the sample by ultrafiltration or dialysis [4].

Native proteins extracted in **Protein Extraction Buffer SuperB** are compatible with protein assays, enzyme activity assays, kinase assays, native and blue native PAGE, and SDS PAGE. Proteins are also compatible with ELISA, immunoprecipitation, and other affinity methods.

Kit Contents

Buffer SB	25 mL
Vial H	0.25 mL
EDTA	0.25 mL

Content Descriptions

Buffer SB	Ready to use Protein Extraction Buffer SB
Vial H	100X Protease Inhibitor Cocktail
EDTA	100X EDTA

Preparation of Extraction buffer for use

Protein Extraction Buffer SuperB Kit **(PN 520112)** is provided in ready-to-use form. Add 10 μL of protease inhibitor cocktail (Vial H) and 10 μL of EDTA (Vial E) to each 1 mL of reagent immediately prior to use. For phosphoproteins, add a phosphatase inhibitor cocktail (not included in the kit) to preserve the phosphorylation state.

Supplied By User

- M220, Covaris S- or E-series instrument with chiller
- Spectrophtometer
- Balance
- 50 mL conical centrifuge tubes
- Centrifuge capable of doing 16000 x g
- Double distilled or 18 megOhm water

AFA Tubes and corresponding AFA Focused-ultrasonicator Holders and Racks:

Description	Part Number
Screw cap microTUBE with AFA fiber	520122
Snap cap microTUBE with AFA fiber	520045
XT microTUBE holder	500358
microTUBE prep station	500330
milliTUBE 1 ml with AFA fiber	520130
XT milliTUBE holder	500348
milliTUBE prep station	500338

Protocol

1. Set the instrument temperature to 6°C. This will help preserve biological activity.

2. Grow cells to a minimum OD_{600} of 1.0 (approximately 3×10^7 cells/mL) [5]. Pellet 40 mL of yeast cell suspension by centrifu**g**ation in a 50 mL conical centrifuge tube at 1000 x g for 4 minutes.

3. Resuspend the cell pellet in 20 mL ddH_2O followed by centrifugation at 1000 x g for 4 minutes. Discard the supernatant.

4. Resuspend the cell pellet in 20 mL ddH_2O containing protease inhibitors and EDTA.

5. Transfer the resuspended yeast cells to a pre-weighed 50 mL conical centrifuge tube.

6. Pellet the cells in the tarred tube by centrifugation at 1000 x g for 4 minutes. Discard the supernatant and weigh the pellet.

7. Resuspend the cell pellet in 770 µL of Protein Extraction Buffer Reagent SuperB for every 100 mg of wet cell mass to yield approximately 1 x 10^8 cells/mL.

8. Mix well to ensure uniform suspension. Dispense 130 µL aliquots into multiple microTUBEs or 1000 µL into an AFA milliTUBE 1 ml. Reserve one tube for a non-AFA control (passive lysis). Using less than a full tube is possible, but there is a risk of splashing portions of the sample to the top of the tube where it might be excluded from the acoustic field.
9. Process the samples using the AFA conditions described in Table 1.

Table 1. Conditions for extracting native proteins from yeast cells

conditions	M220		S220/E220	
	microTUBE	milliTUBE	microTUBE	milliTUBE
volume	130 µL	1000 µL	130 µL	1000 µL
cell number	10^6-10^7	10^8	10^6-10^7	10^8
peak incident power (PIP)	75W	75W	80-100W	80-100W
duty factor	10%	10%	10%	10%
cycles per burst (CPB)	200	200	200	200
time (seconds)	180	180	180	180
temperature	6°C	6°C	6°C	6°C
degassing mode	NA	NA	continuous	continuous

10. Following AFA treatment, transfer the sample from the AFA tube to a clean microfuge tube. Centrifuge at 16,000 x g for 10 minutes and collect the clarified supernatant for analysis.

Appendix A

Protein Concentration Determination

The amount of protein can be determined using the following techniques:

1. UV at 280 nm
2. Bicinchoninic acid (BCA) method
3. Bradford method
4. Lowry method

Each method has its advantages and disadvantages, and the user should use a methodology that is currently used in their laboratory or a method that fits into their workflow.

Appendix B

Analyses After Extraction

Once the sample is processed and clarified it can be analyzed using a variety of techniques such as:

1. Elisa
2. Specific protein activity assay
3. Further fractionation using SDS PAGE, capillary electrophoresis, 2-dimension gel electrophoresis or any number of separation matrices using low and/or high pressure chromatography (HPLC)
4. Tryptic digestion followed by reverse chromatography and mass spectrometry.

References

1. Collins T, D'Amico S, Georlette D, Marx, JC, Huston, A.L, Feller, G. *Anal. Biochem*., 352 (2006) 299–301.
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3. Goldberg MF, Expert-Bezancon N, Vuillard L, Rabilloud T. *Fold. Des*. 1 (1996) 21-27.
4. Smejkal GB. *Expert Rev. Proteomics*, 10, (2013) 407-409.
5. Day A, Schneider C, Schneider BL. Yeast Cell Synchronization, Methods Mol. Biol. 241 (2004) 55-76.