

# Active Extraction of Native Proteins from Yeast using Covaris Adaptive Focused Acoustics® (AFA®)

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## Abstract

Using yeast as a model organism, several commercially available native protein extraction reagents were tested for total protein yield and preservation of enzymatic activity using either a “passive extraction” method or an AFA-based “active extraction” method. With every buffer evaluated, AFA-based active extraction increased both total protein yield and enzyme activity over the manufacturer’s passive extraction protocol.

- Three-fold increase in total protein was observed when the Yeast Protein Extraction Reagent (Y-PER™) when used with AFA
- Two-fold increase in total protein yields from the YeastBuster™ Protein Extraction Reagent (YB) when used with AFA
- In addition, the Covaris SuperB reagent exhibited 6.6 times more phosphomonoesterase activity than Y-PER and 1.5 times more activity than the YB reagent

## Introduction

The significant mechanical strength of the yeast cell wall of *Saccharomyces cerevisiae* makes the recovery of biologically active proteins particularly challenging. Even though the cell wall constitutes 10 to 25% of the total yeast cell mass [1] and cell wall proteins constitute roughly 40% of the cell wall mass [2], total protein yields from yeast are typically very low. Conventional mechanical techniques to improve cell lysis (e.g., bead beating), typically employ rigorous agitation that simultaneously drives both protein denaturation and the loss of protein activity.

Alternatively, the use of harsh chemicals to improve cell lysis can also radically alter protein molecular mass and functionality. For example, the transmembrane insulin receptor at 460 kDa binds enough Triton X-100 detergent to inflate its putative mass to over 1,000 kDa [3]. In addition, the high micellar molecular weight of some detergents also makes them difficult to remove for downstream analyses. Tween 20 is commonly used at concentrations over six times its critical micelle concentration where over 85% of the detergent mass exists as 38 kDa micelles [4].

The dilemma is that the physiological buffers used to maintain proteins in their native state are “mechanically” inert, and therefore, ideally would require a mechanical process to disrupt cell walls and organelle membranes without modifying proteins

or driving protein aggregation or precipitation. The non-contact, isothermal, and the hydrodynamic shear force “mechanical” properties of AFA have been shown to be highly effective for cell and tissue disruption, and significantly improving the compatibility of downstream analytical methods by lowering the concentration, and in some cases, eliminating the need for detergent use. This note describes the use of AFA-based lysis of yeast cells and protein extraction.

## Materials and Methods

### Yeast Cultures

A mass of 0.35 g of dried active Baker’s yeast (ConAgra, Naperville, IL) was hydrated in 40 mL of 80 mM sucrose and incubated for three hours with shaking at 300 rpm at 20 °C. The cells were pelleted by centrifugation at 800 x g for one minute, washed in 40 mL H<sub>2</sub>O, and pelleted again. Cells were resuspended in 20 mL H<sub>2</sub>O and an aliquot was diluted 1:1000 in PBS for cell counting using the Scepter 2.0 Automated Cell Counter (Millipore, Danvers, MA). Halt™ and EDTA protease inhibitors (Thermo Scientific Pierce Biotechnology, Rockford, IL, USA) were added to the second wash and the cells were pelleted by centrifugation at 1000 x g for two minutes. Cells were resuspended to a final concentration of 10<sup>9</sup> cells/mL in each reagent.

## Reagents for Native Protein Extraction from Yeast

The Yeast Protein Extraction Reagent (Y-PER) was purchased from Thermo Scientific Pierce (Rockford, IL, USA). The YeastBuster (YB) Protein Extraction Reagent and tris (hydroxypropyl) phosphine (THP) were purchased from EMD-Millipore (Danvers, MA, USA). The YB reagent contained 5 mM THP as per manufacturer's recommendation. The Covaris SuperB protein extraction reagent (Woburn, MA, USA) was tested with or without 5 mM THP. All reagents were supplemented with the protease inhibitor cocktail.

## Sample Processing

One milliliter of cell suspension was dispensed in a Covaris milliTUBE and processed in a Covaris S220 Focused-ultrasonicator using a setting of 80W peak incidence power (PIP), 10% duty cycle (DC), and 200 cycles per burst for 180 seconds at a temperature of  $20.7 \pm 0.5$  °C. Each reagent included a separate control tube which was incubated at room temperature according to manufacturer's instructions. Following AFA, cell lysates were centrifuged at 21,000 x g for 10 minutes and the supernatants were aspirated for further analyses.

## Protein, DNA, and Enzyme Activity Assays

Protein concentrations were determined using the Quick Start™ Bradford Protein assay (BioRad, Hercules, CA). Total DNA was quantified using the Qubit™ assay (Invitrogen, Carlsbad, CA). Phosphomonoesterase activity was measured using the Total Phosphatase Assay Kit from G-Biosciences (St. Louis, MO, USA). Lysates were diluted in phosphatase assay buffer supplemented with 10 mM MgCl<sub>2</sub> and incubated with p-nitrophenyl phosphate substrate for 30 minutes at room temperature. The reactions were stopped with 3M NaOH and p-nitrophenolate measured at 405 nm.

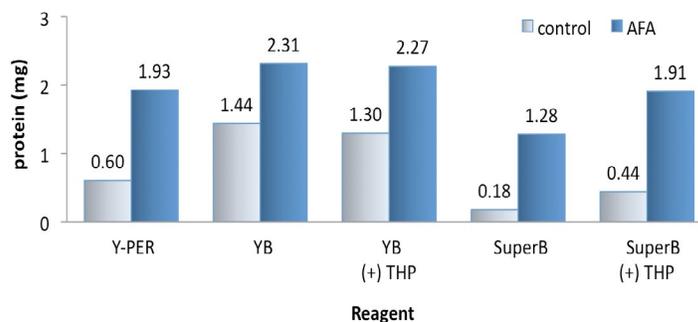
Following the addition of 5 mM CaCl<sub>2</sub> to the samples, endopeptidase activity was restored as measured by the hydrolysis of the synthetic peptide benzoyl-DL-arginine p-nitroaniline hydrochloride (BAPNA) and the release of free p-nitroaniline at 405 nm as described by Oppert et al. [5]

## Results and Discussion

### Total Protein from Yeast Cell Lysates

For all reagents tested, the amount of total protein recovered in supernatants was significantly increased when AFA-based active extraction was used for cell lysis. The YB reagent yielded the highest total protein of the tested reagents, 17.9% and 19.3%

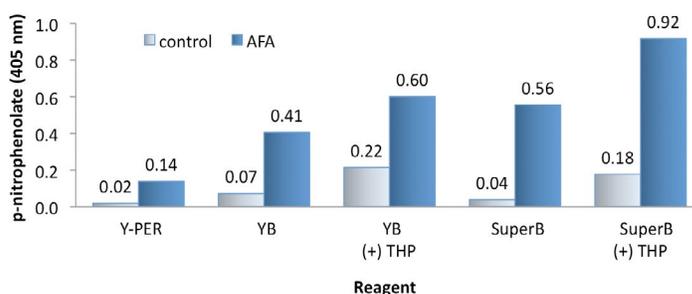
more protein than Y-PER and SuperB, respectively (**Figure 1**).



**Figure 1: Total protein yields from yeast.** Total protein yields in yeast cell lysates prepared with or without AFA-based active extraction in Y-PER, YB, and SuperB. The YB and SuperB reagents were tested with 0 or 5 mM THP.

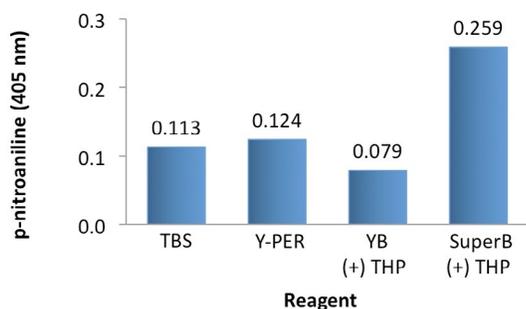
### Enzyme Activity

For all reagents tested, AFA-based active extraction yielded more phosphatase activity than reagent controls. The SuperB reagent used in combination with AFA-based active extraction yielded 14.6 times more activity than reagent controls. SuperB with 5 mM THP yielded 6.6 and 1.5 times more total phosphatase activity than Y-PER and YB, respectively (**Figure 2**). SuperB without THP recovered nearly identical phosphatase activity per unit of cell mass as the YB reagent. Compared to the Y-PER and YB reagents which contain detergents, the SuperB Reagent contains a proprietary non-detergent sulfo betaine (NDSB). Unlike detergents, NDSB does not form micelles and are easily removed from the sample prior to downstream analyses. Moreover, NDSB has been shown to prevent protein precipitation, increase the yields of membrane and cytoskeletal proteins, and improve the recovery of active enzymes [6,7].



**Figure 2: Total phosphate activity.** Total phosphatase activity in yeast cell lysates prepared with or without AFA-based active protein extraction in Y-PER, YB, and SuperB. Data was normalized to initial cell mass. The highest enzymatic activity was recovered using the SuperB reagent with 5 mM THP. The YB and SuperB reagents were tested with 0 or 5 mM THP.

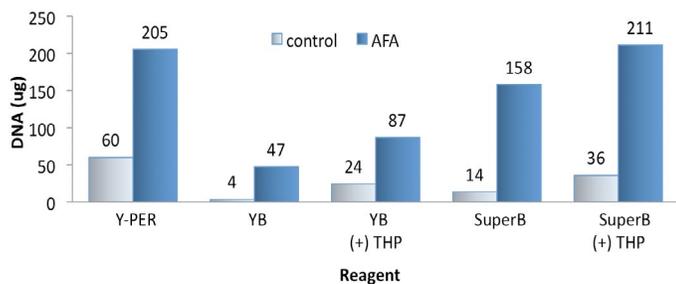
Hydrolysis of the BAPNA substrate showed a two-fold retention of endopeptidase activity in SuperB extracts compared to Y-PER and YB extracts, which were similar to TBS extracts (**Figure 3**).



**Figure 3: Endopeptidase activity.** Endopeptidase activity measured in cell lysates prepared with TBS, Y-PER, YB, and SuperB using AFA-based active protein extraction. Activity was determined from the hydrolysis of the synthetic trypsin substrate BAPNA over 30 minutes and the release of p-nitroaniline measured at 405 nm. Residual activity in Y-PER and YB lysates were similar to TBS lysates. Twice as much activity was preserved in SuperB lysates.

### Disruption of Nuclei Under Native Conditions

DNA was measured as an indirect monitor of the efficiency at which nuclei were disrupted under native conditions. Regardless of the extraction reagent used, greater amount of DNA was recovered from active AFA-based treated samples. These data suggest that Y-PER and SuperB, when used in conjunction with AFA, disrupted nuclear membranes more effectively than YB (Figure 4). Effective disruption of nuclei is critical to comprehensive proteomics analysis, since 27% of the 6,100 known yeast proteins are nuclear proteins [8].

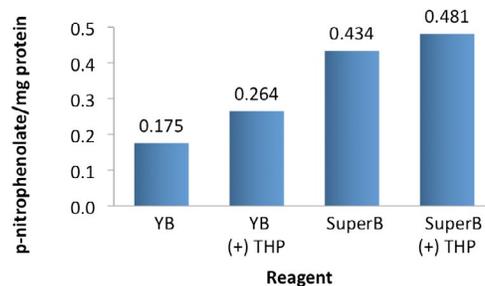


**Figure 4: DNA yields from yeast cell lysates.** DNA yields in yeast cell lysates prepared with or without AFA in Y-PER, YB, and SuperB. DNA was measured by Qubit DNA assay. The YB and SuperB reagents were tested with 0 or 5 mM THP.

### Effects of THP on Cell Lysis Efficiency

The inclusion of a reducing agent is advantageous for yeast cell lysis, since disulfide linkages are a predominant feature of cell wall proteins [9]. Both YB and SuperB reagents contained 5 mM of the reducing agent THP. The Covaris reagent SuperB supplemented with 5 mM THP increased total protein yield 48% over SuperB without THP.

When normalized to protein mass, the relative phosphatase activity in SuperB and YB reagents were similar with or without THP. While YB had yielded the most total protein, phosphatase activity relative to protein mass was lower than SuperB (Figure 5). These data suggest that THP contributes to the increase in total protein, and hence, more total phosphatases, and the proprietary detergent in the YB reagent, while good for releasing more total protein, lowers the recovery of active proteins.



**Figure 5: Normalized phosphate activity.** Phosphatase activity expressed as p-nitrophenolate product per milligram of protein mass showing the effects of THP reducing agent added to the YB and SuperB reagents.

### Conclusions

Compared to the passive diffusion methods of protein extraction routinely used in proteomics, the active turbulence AFA-based extraction method increased total protein and recoverable enzyme activity in all the native protein extraction reagents tested. While the Covaris SuperB reagent yielded comparable total protein than the Y-PER and YB reagents, the reagent was superior for the recovery of biologically active proteins. Covaris SuperB reagents also yielded the highest amount of DNA suggesting a more complete disruption of the cells and its organelles.

As the analytical methods of proteomic analysis have become more sensitive, it has opened the field for the utilization of limited and challenging sample types. The pre-analytical sample preparation significantly benefits from the use of advanced and active extraction methods such as Covaris AFA along with compatible reagents to ensure effective and robust protein extraction from these challenging samples.

## References

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