

Yeast Lysis Application Note:

Introduction:

The yeast cell as a model eukaryotic system has and continues to provide much insight and value in genetic, biochemical, and drug discovery research. Yeast is of popular use for recombinant protein production and as an expression system due to its unicellular nature combined with its structural and functional aspects, which have many similarities to higher eukaryotes, e.g., the ability to perform post translational modifications to proteins. Yeast cells have been heavily researched so advantages include the availability of a complete genomic sequence and research tools like an assortment of vectors for recombinant protein expression. Furthermore, as compared to mammalian cells yeast grow in simple media, grow relatively rapidly, and to a high density⁽¹⁾.

The main issues associated with yeast utilization and research typically involves determining a method for efficient cell lysis and protein extraction. This is because *Saccharomyces cerevisiae*, the most often employed yeast, is surrounded by a thick, tough, and rigid cell wall which prevents easy extraction of the desired intracellular products⁽²⁾. Traditional extraction techniques often involve harsh and extreme conditions which could potentially damage desired products or limit yields. Enzymatic digestion can be expensive when processing large amounts of samples and the enzymes used to attack the yeast cell wall often make it difficult to obtain the desired products i.e., mRNA or recombinant proteins, in their native and intact form⁽³⁾. Furthermore, a second step is often required to lyse spheroplasts generated from the commonly used digestion enzymes (zymolase, glucalase and/or lyticase). The most popular mechanical methods used to lyse yeast, glass beads, often result in significant protein denaturation and/or loss of extracted proteins that non-specifically bind to the glass beads. In addition, mechanical methods such as this can be cumbersome when processing large amounts⁽⁴⁾ and are not suited to the high-throughput format required in today's world. Sonication has been a widely used, successful method for cell disruption⁽⁵⁾ due to its speed, ease, and ability to lyse a range of cells, but until now has only been relevant to single-sample techniques. The SonicManTM continues the traditional method of cell lysis with sonication and extends it into the high-throughput format.

The SonicMan (Figure 1) offers the ability to lyse a variety of cells with configurable settings allowing for lysis of easily disrupted cells (i.e., insect or mammalian cells), to difficult to disrupt cells (i.e., *E. coli* cells), and to highly difficult to disrupt cells (i.e., yeast cells⁶).

Mechanism of Cell Disruption by Sonication

Cell membrane disruption by sonication is directed by ultrasound induced cavitation. Ultrasonics propagates in liquid mediums by pressure waves that alternatively expand and contract and in so doing, create microbubbles or 'cavities.' Collapse of these cavities can produce extreme shear forces with the ability to disrupt membranes.⁷⁻¹⁰

Figure 1A:

96 disposable pinned lids.
384 pinned lids also available. The lids provide the means to scale up sonication to high-throughput processes. Custom labware upon request.

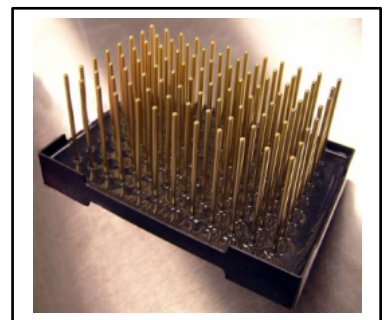


Figure 1B:

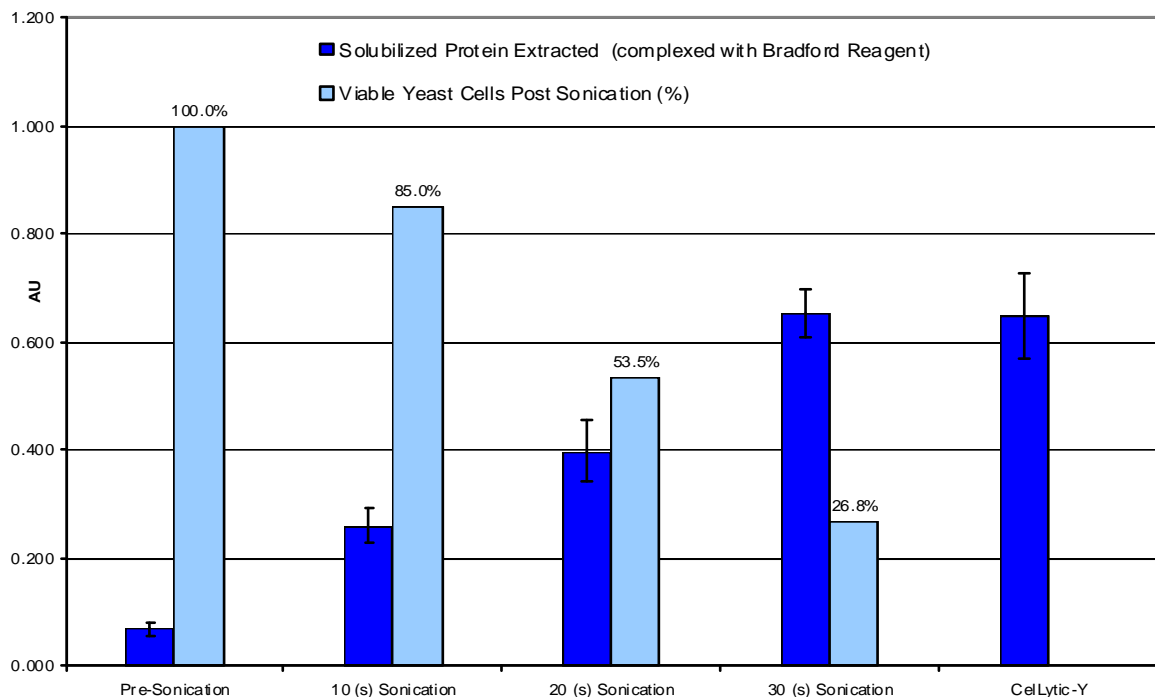
Front view of the SonicMan with touch screen panel



Yeast Culturing

A 50 mL O/N culture of *Saccharomyces cerevisiae* (Fleischmann's Bakers Yeast) was grown in Yeast extract Peptone Dextrose (YDP) broth overnight (16 hours) in a 30°C water bath shaker. Fresh YDP media (250 mL) was inoculated with 1 mL of the O/N culture and grown for 4 hours to an OD₆₀₀ of between .700-.800 (log phase). The cells were collected with centrifugation (Beckman, 2500g, 10min, 4°C), washed with chilled PBS buffer, and re-suspended in 50 mL of chilled PBS buffer. Cell viability was determined after each sonication time (see sonication settings, below) by retrieving an aliquot and diluting appropriately for a serial dilution method.

Yeast Cell Lysis/Protein Extraction



Sonication Settings:

300 μ L aliquots of re-suspended yeast cells were transferred to 1.4 mL polyethylene tubes (Matrix, Hudson, NH) and the tubes placed in their corresponding 96-well-form at Matrix tube rack. The plates were subjected to sonication times of 0, 10, 20, 30 seconds at 100% power (\approx 12 watts/pin). The samples were then centrifuged (Galaxy 7, VWR, West Chester, PA) at 8000 rpm for 15 minutes to pellet debris. The supernatant containing solubilized proteins was collected for analysis.

CelLytic-Y Protocol:

300 μ L aliquots of re-suspended yeast were centrifuged (Galaxy 7, VWR, West Chester, PA) at 2500g for 10 min to pellet cells. The cells were re-suspended in 20 μ L (5mL/g pellet as recommended by Sigma protocol¹⁰) of CelLytic-Y Yeast Cell Lysis/Extraction Reagent (Sigma-Aldrich Chemical Company, St. Louis, MO) and gently shaken for 30 minutes corresponding to the Sigma protocol. 280 μ L of PBS buffer was added to the solution post mixing (to normalize the solution with the sonicated samples) and the solution centrifuged to pellet debris and the supernatant collected for analysis.

Protein Assay:

The amount of protein released after each sonication time and CelLytic-Y incubation was qualitatively determined by use of Bradford Reagent (Sigma-Aldrich Chemical Company, St. Louis, MO). An aliquot of 100 μ L of each sample was mixed with 900 μ L of Bradford Reagent and the absorption at 595 nm recorded (Shimadzu UV-1601 UV-Visible spectrometer) after 10 minutes of mixing time.

Results:

The efficiency of cell disruption was quantified by the amount of soluble protein released determined by mixing with Bradford Reagent (Sigma-Aldrich, St. Louis, MO). The results indicate that at 100% sonication power the maximum protein release occurs at 30 seconds of sonication. As expected, protein release is inversely correlated with cells left intact. The SonicMan results were comparable with CelLytic[™]-Y Yeast Cell Lysis/Extraction Reagent (Sigma-Aldrich, St. Louis, MO) in their respective abilities to release proteins.

Instrument Details

- **Stand-alone or integrated bench-top instrument**
- **Interchangeable 96, 384, and 1536 format disposable pinned lids (custom labware upon request)**
- **Plate shuttle which allows for direct integration with pick and place robotics**
- **Touch screen interface**
- **Variable power settings between 1 and 1,150 Watts**
- **Variable sonication time intervals from 0.1 to 20 seconds.**

SonicMan Benefits:

- **Speed:** The SonicMan can efficiently lyse cells in seconds significantly faster than most other cell disruption methods.
- **Variability & Configurability:** The SonicMan has configurable power and time settings allowing for power outputs capable of lysing a range of cells from mammalian cells to yeast cells.
- **Clean & Easy:** No specialized reagents needed meaning no post enzyme cleanup and easy carry over to downstream applications.
- **High-throughput:** The SonicMan brings the highly used sonication cell disruption method to the high-throughput era.

References:

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<http://www.sigmaaldrich.com/sigma/bulletin/c4482bul.pdf>