

Bacterial Lysis Application Note

Introduction:

A method to disrupt or lyse cells is invariably required in the research and production of intracellular products in the biochemical and biomedical industries. Requirements for cell lysis include speed (to avoid further biochemical changes), configurability (disruption power capable of lysing a range of membrane strengths, i.e., mammalian cells to yeast cells), and integration (ease of carryover from upstream applications and to down stream applications). Traditionally, cells have been lysed by non-mechanical methods, mechanical methods, or even using a combination of both. Non-mechanical methods such as detergents, alkali, and enzymatic degradation are typically selective and often require additional reagents and/or a removal process to continue on to downstream applications. Mechanical methods of cell disruption are often cumbersome, irreproducible, time-consuming, and prone to cross-contamination and as such are not suitable for high-throughput formats. Sonication has been a widely used, successful method for cell disruption³ due to its speed, ease and cleanliness, and ability to lyse a range of cells but until now has only been relevant to single-sample techniques. The SonicMan™ continues the traditional method of cell lysis with sonication and extends it into the high-throughput format. The SonicMan 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 cells⁴).

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.⁵⁻⁸

Figure 1A:

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

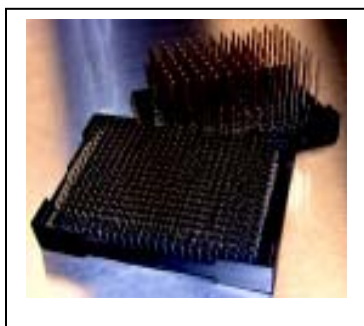


Figure 1B:

Front View of the SonicMan with touch screen panel

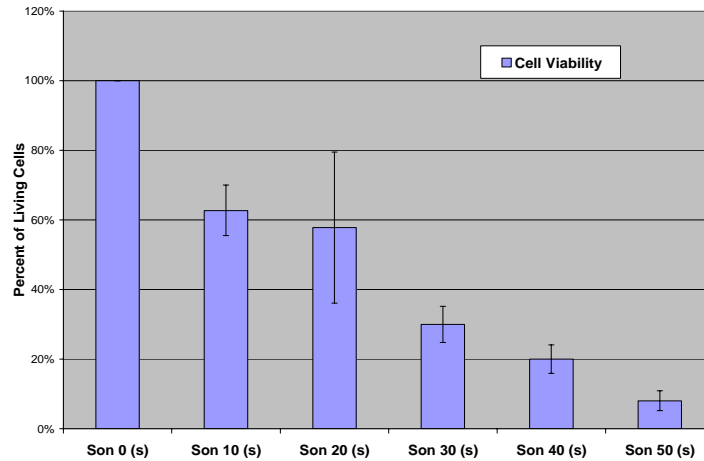


The SonicMan

- 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.
- Gasketed pin lids to ensure sample integrity with no well to-well cross contamination

Data:

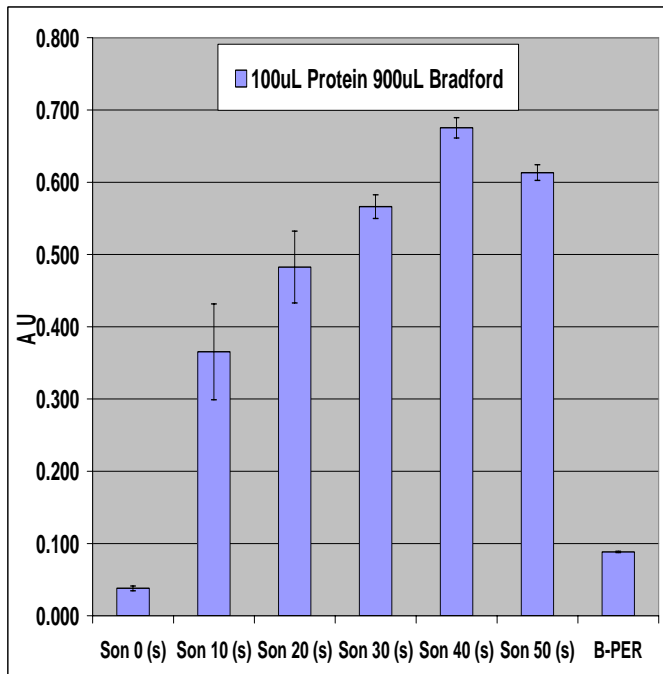
Figure 1: Cell Viability



Bacteria Culturing

A 50 mL culture of *Escherichia Coli*. BL21 (DE3) was grown in Luria-Bertani (LB) media overnight (16 hours) in 37°C water bath shaker. A fresh 250 mL LB culture was inoculated with 1 mL of these cells and grown for 4 hours to an OD₆₀₀ between .600 and .700. The cells were collected with centrifugation (Beckman, 1000g, 10min, 4°C), washed twice 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, Fig. 2) by retrieving an aliquot and diluting appropriately for a serial dilution method.

Figure 2: Protein Quantification



Sonication Settings:

300 uL aliquots of re-suspended bacteria were transferred to 1.4 mL polyethylene tubes (Matrix, Hudson, NH) and the tubes placed in their corresponding 96-well-format Matrix tube rack. The plates were subjected to sonication times of 0, 10, 20, 30, 40, and 50 seconds at 100% power (≈ 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.

B-Per Protocol:

300 uL aliquots of re-suspended bacteria were centrifuged (Galaxy 7, VWR, West Chester, PA) at 1000g for 10 min. to pellet cells. The cells were resuspended in 60 uL of B-Per Reagent (Pierce, Rockford, IL) and vortexed for 1 minute corresponding to the Pierce protocol. 240ul of PBS buffer was added to the solution (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 was qualitatively determined by use of Bradford Reagent (Sigma-Aldrich Chemical Company, St. Louis, MO). An aliquot of 100 uL of each sample was mixed with 900 uL 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 40 seconds of sonication. As expected, protein release is inversely correlated with cells left intact. The SonicMan compared favorably with Pierce's B-per protein extraction reagent in their respective abilities to release proteins.

SonicMan Benefits:

- **Speed:** The SonicMan can efficiently lyse cells in seconds dramatically 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 carryover 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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