





APPLICATION NOTE

Lysing of Bacterial Cells in the Geno/Grinder

With kind permission of Russell H. Vreeland, West Chester University, January 2003

The SPEX CertiPrep 2000 Geno/Grinder was tested to determine if this technology could be used to lyse bacterial cells. Standard 96-well titer plates were used with 400-600 μ silica grinding beads (Molecular Biology Grade, cat. no. 2166).

The delivery of the beads into each cell of the titer plate can be accomplished in a number of ways. In this case micropipette tips were filled to the mark with grinding beads, and each tip emptied into a titer plate well. This technique will deliver approximately 0.4 grams of silica beads per well. Other delivery systems are commercially available.

Experiments were performed with 250 - 500 ml of bacteria: in one case the gram-negative, salt-tolerant *Halomonas elongata*, and in the other, gram-positive *Bacillus*. Both organisms were grown to late log state at 37° C, with shaking, then harvested via centrifugation at 7000 RPM for 10 minutes. Cells were washed once with sterile 8% (w/v) NaCl solution to remove spent growth medium. After washing, the cell pellet was suspended in 20 ml of sterile 8% NaCl solution. Each well of the test plate was filled with 0.5 ml of this suspension; it was important that the cells to be lysed were present in high concentration. 0.4 grams of silica beads were added to each well, and the plate was sealed. The filled titer plates were then shaken in the Geno/Grinder at a setting of 1450 strokes per minute, in one-minute intervals for periods ranging from one minute to twelve minutes. Culture filtrate from the shaken plates was then measured for optical density at 260 nm. The average data for three separate "well sets" is shown in Figure 1.

Conclusion

The 2000 Geno-Grinder is capable of lysing bacterial cells in 96-well titer plates with 400-600 μ silica beads as grinding media. With increased grinding time culture filtrate shows a definite increase in optical density, indicating release of nucleic acids during grinding. Grinding times of 6 to 9 minutes appear to be suitable for producing measurable amounts of nucleic acid.

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:: APPARATUS:

Geno/Grinder[®]

:: APPLICATION: DNA/RNA and Other Extractions



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