Evaluating Five Microplate Mixing Techniques: Diffusion, Centrifugation, Shaking, Pipetting and Ultrasonic Mixing

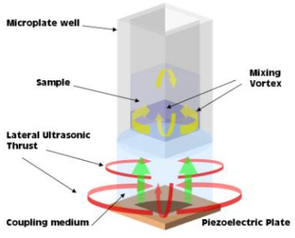
Jean Shieh, Bruce Jamieson and Vibhu Vivek; Microsonic Systems Inc.

Abstract

Drug discovery miniaturization efforts have been successful in dramatically increasing the density of microplate assays and substantially reducing assay volumes. Unfortunately, these improvements have increased the physical difficulties associated with adequate mixing of assay components. As microplate well volumes decrease, variables such as surface tension and the aspect ratio of taller, thinner wells have raised concerns about the effectiveness of traditional mixing techniques. To address this, Microsonic Systems has developed the HENDRIX SM100 specifically for mixing, solubilization and suspending liquids in 96, 384, 1536 well formats and beyond. Microsonics has recently developed a methodology to evaluate the thoroughness of microplate assay mixing and used it to characterize the effectiveness of mixing in 384 well microplates with five common mixing techniques: diffusion, centrifugation, shaking, pipetting as well as ultrasonic mixing with our own HENDRIX SM100. The method is simple, yet precise and accurate and uses standard drug discovery tools such as single- and multi-channel pipettes, an automated liquid handler and a UV/Vis microplate spectrophotometer. This poster describes the method and results of these five mixing alternatives.

Technology Overview

Microsonic Systems' patented Lateral Ultrasonic Thrust™ (LUT™) technology works by using a Micro-Electrical-Mechanical Systems (MEMS) based transducer, which when excited with RF power generates ultrasonic waves. These ultrasonic waves pass into the sample as broad beams of acoustic energy. The energy creates regions of strong Lateral Ultrasonic Thrust that in turn creates strong mixing in the form of a rapid vortex. LUT technology, unlike other ultrasonic methods, does not cause cavitation. At high power, LUT technology can be used for solubilization and thawing applications, and at low power, the same technology can be used for assay mixing or bead suspension.



The **HENDRIX SM100 Ultrasonic Fluid Processor** utilizes LUT technology to solubilize compounds and recover precipitated samples. The same system is also used for HTS assay mixing, thawing frozen tubes and plates and bead suspension applications.

The HENDRIX SM100 comprises a Fluid Processor Unit (FPU) and a Base Control Unit (BCU). The FPU processes samples in various densities, from 24-vial & 96-tube racks to 3456-well formats. The BCU houses the main control system and a Peltier chiller for keeping the coupling fluid at a constant temperature.

Material and Methods

1. Microplates (Greiner Cat# 781280) were mapped to allow various well volumes to be sampled automatically by means of a robotic liquid handler ("Biocross" ADS-384-8, from B.T.C., Japan).
2. Two microliters of Bromophenol Blue (Spectrum Chemical # BR144) in DMSO (Research Organics, Inc. Cat# 2164D) at concentration of 12mg/mL was layered under 48 µL of water in each well using the Biocross. The final volume in each well was 50 µL.
3. Five mixing methodologies were employed on each plate (see **Table 1** for conditions) and then sampled.
4. The Biocross was programmed to sample 9 µL at various heights, starting at the top of the well and working downward; a total of five aliquots were collected from each well.
5. Aliquots were then diluted (1:10) into a 96-well format and read in a UV/Vis Microplate reader (Tecan Sunrise)
6. Differences in gradient absorbance readings for different aliquots were recorded, and the % coefficient variation (%CV) between the aliquots was calculated for each mixing technique in order to measure the mixing effectiveness.

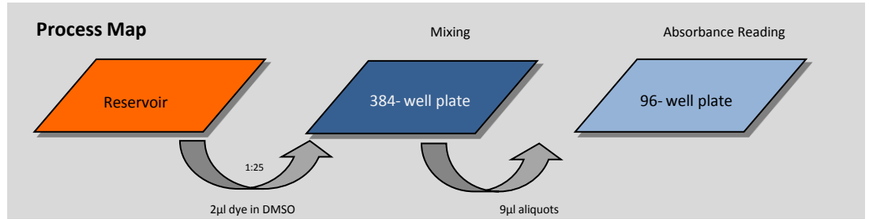


Table 1. Mixing Technique and Conditions

Mixing Technique	Conditions Used
Lateral Ultrasonic Thrust™ (HENDRIX SM100)	Power- 38 V Duty cycle- 50% Rep rate- 1000 Hz 25 and 50 cycles
Pipetting (Biocross)	3 repetitions of aspirate and dispense, with and without vertical movement
Centrifugation (Sorvall, RT6000)	2000 rpm for both 1.5 minutes and 5 minutes
Orbital Shaker (INHECO Gmbh)	20 minutes of mixing
Diffusion	60 minutes of mixing

Results

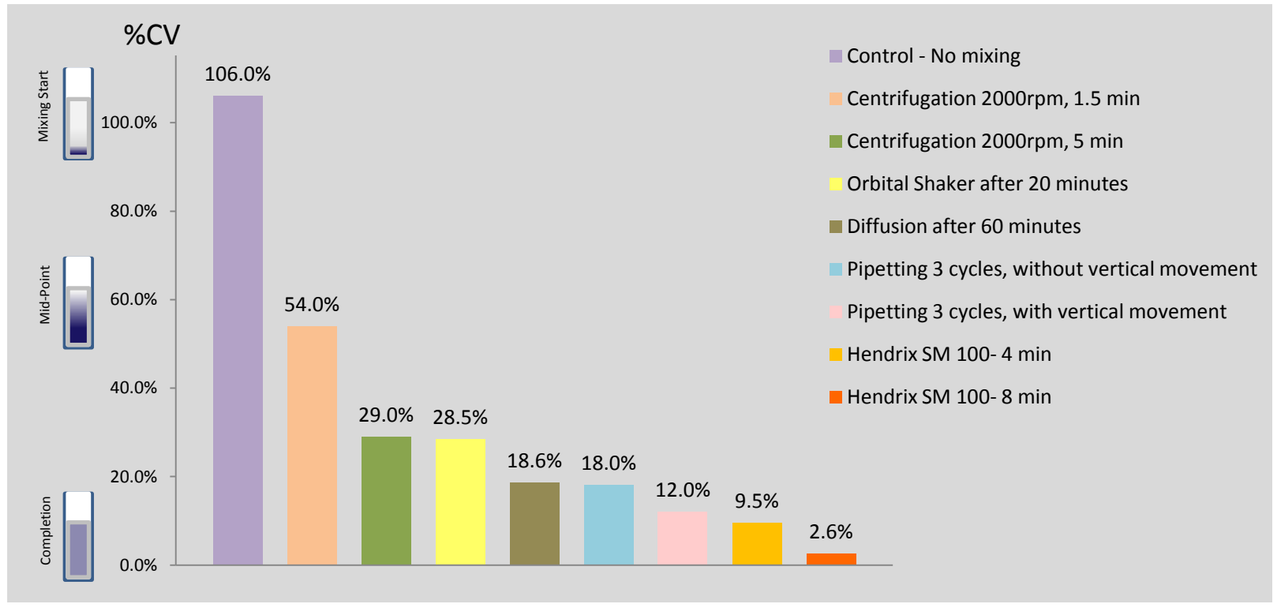


Figure 1. When we examined the mixing effectiveness of the five methods, spinning plates in the centrifuge for 90 seconds was ineffective (54% CV) and even after 5 minutes centrifugation could not fully mix the wells (29% CV). Shaking plates for 20 minutes was also ineffective (28.5% CV). Mixing by diffusion took 60 minutes to reach 18.6% CV; pipetting repeatedly did reduce the %CV but contacting samples could introduce other issues. The most effective way to mix a small volume of a DMSO sample into water in 384-well plates was using non-contact Lateral Ultrasonic Thrust technology. The HENDRIX SM100 achieved 9.5% CV in 4 minutes and 2.6% CV in 8 minutes.

Summary

Table 2. Summary of Mixing Technique Benefits and Cost

Mixing Technique	Benefits	Costs/Disadvantages
Lateral Ultrasonic Thrust™ (HENDRIX SM100)	1. Stand-alone instrument 2. Fast and easy to use 3. Non contact- no cross contamination 4. Automatable for 96,384,1536, 3456-well format	Initial acquisition cost but minimum operating cost
Pipetting	May already be part of the process	1.Requires consumables or risk of contamination 2.Not applicable to 1536 and 3456-well formats 3.Lengthy protocol creation
Centrifugation	Typically available in labs	Not an effective technique for mixing
Orbital Shaker	Typically available in labs Low acquisition cost	1.Time consuming 2.Not optimized for higher density plates with smaller wells
Diffusion	No equipment required	Time consuming and not an effective technique for mixing

Conclusion

Interest in microplate-mixing is growing due to developments in life sciences, agricultural sciences, analytical chemistry and food engineering. The homogeneity of an assay solution has profound effects on the results of the assay. This study shows that HENDRIX SM100 is the most rapid and efficient technique to achieve a well mixed state in smaller assay volumes. Mixing with a shaker requires more time than is practical, and centrifugation and diffusion are ineffective. Pipetting could enhance mixing but it cannot be scaled down to 1536 & 3456-well formats. Therefore, the HENDRIX SM100 is the clear choice for low-volume assay mixing.

Acknowledgments

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MICROSONIC SYSTEMS™
76 Bonaventura Dr.
San Jose, CA 95134
www.microsonics.com