Mixing Effectiveness-A Methodology and Study of Microplate Mixing Techniques Including Ultrasonic HENDRIX SM100

Mike Travis[1], Vibhu Vivek[1], Bruce Jamieson[1], Jean Shieh[1] and Dave Weaver[2]
[1]Microsonic Systems, [2]Vanderbilt University

Introduction

Miniaturization efforts have been successful at dramatically increasing the density of microplate assays, substantially reducing assay volumes but inadvertently adding physical difficulties regarding adequate mixing of the 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 mixing with traditional shakers. To address this, Microsonic Systems has developed the HENDRIX SM100 specifically for mixing, solubilization and suspending beads in 96-, 384-, 1536-well formats and beyond.

Evaluating the thoroughness of microplate assay mixing is not yet well characterized with a proven testing methodology. Microsonics has recently developed such a methodology and used it to characterize the effectiveness of mixing in 384-well microplates with some common mixing techniques such as diffusion and mechanical mixing as well as our own Lateral Ultrasonic Thrust™ technology. This poster will describe the method and results of these three mixing alternatives. The method is simple yet precise and accurate and uses standard drug discovery tools such as single and multichannel pipettors, an automated liquid handler and a UV/Vis microplate spectrophotometer. The results conclusively show that the mixing achieved by Lateral Ultrasonic Thrust utilized by the HENDRIX SM100 is far more effective than either diffusion or mechanical mixing.

Method

Microplates (Greiner cat. # 781280) were mapped to allow the various well volumes to be sampled automatically by means of a robotic liquid handler (“Biocross” ADS-384-8 , from B.T.C., Japan), with one plate per time point. That is, four replicate wells were filled at each of the total volumes to be studied, on a single 384-well plate for each sampling time. Bromophenol Blue (Spectrum Chemical # BR144) in DMSO was manually introduced to the bottom of each well by a hand-held, single channel pipettor, 2 µL in each well. No mixing through the sample introduction was observed. The three mixing methodologies studied were employed on each plate and then sampled.

The Biocross was programmed to sample 9 µL at various heights, starting at the top of the well and working down. This yielded discrete samples from decreasing heights from top to bottom of each well; the aliquots were labeled as sample selection 1 to N, as shown in Figure 1. These samples were then diluted (1:50) into a read-out plate and read in a UV/Vis Microplate reader (Tecan Sunrise).

Figure 1. Side view of a well in the 384-well sample plate; aliquots are taken at each time sampling point. Sample sections are numbered from 1 to N (1 to 5 for the 50 µL fill volume sample well as illustrated) from well bottom to well top.

Results

We observed gradient absorbance readings for the different sample sections when mixing first started, with the highest reading observed in sample section #1 which was the aliquot from the bottom of the well and the lowest reading observed from the top of the well. As mixing continued, the difference in absorbance readings among the sample sections reduced and eventually the well reached mixed condition when the curve was nearly flat. However, the elapsed time to this mixed condition varied from one mixing technique to another. Figure 2 shows that at 50 µL sample volume in the 384-well microplate, the HENDRIX SM100, utilizing the mixing technique known as Lateral Ultrasonic Thrust, reached a mixed condition after 8 minutes of mixing while the orbital shaker required 60 minutes to reach the same condition and diffusion took 120 minutes to mix.

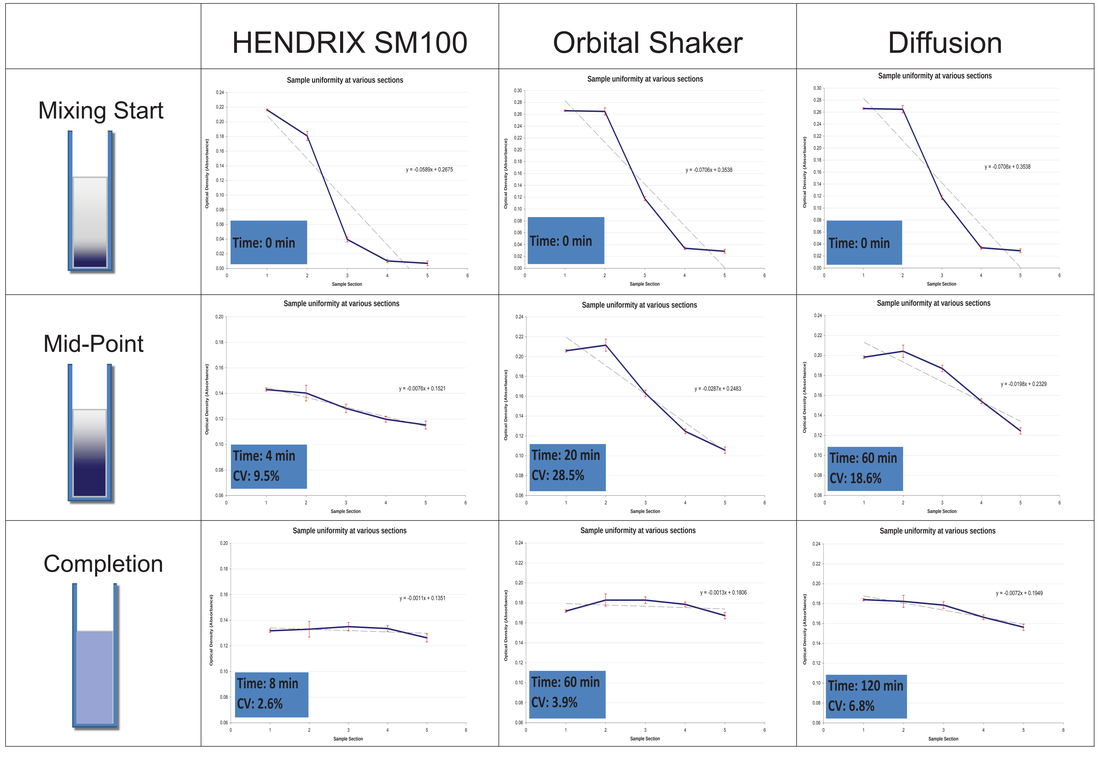

Figure 2. Comparison of three mixing techniques – Lateral Ultrasonic Thrust™ technology (HENDRIX SM100), mechanical mixing (orbital shaker) and diffusion – are shown for total volume at 50 µL. Three time points are shown for each technique, mixing start, mid-point and completion. The HENDRIX SM100 reaches completion with the shortest elapsed time and the lowest %CV.

Summary

As summarized in Figure 3:

1. For all three mixing techniques, longer mixing time was required as the well volume increased.
2. In the entire well volume range studied, samples mixed by HENDRIX SM100 reached mixed conditions around 7X faster than samples mixed by orbital shaker and 12X faster than samples mixed by diffusion.
3. Both the orbital shaker and diffusion mixing methods were not sufficient to mix dye-dosed DMSO into water in reasonable times.
4. The HENDRIX SM100 was able to mix samples in minutes.

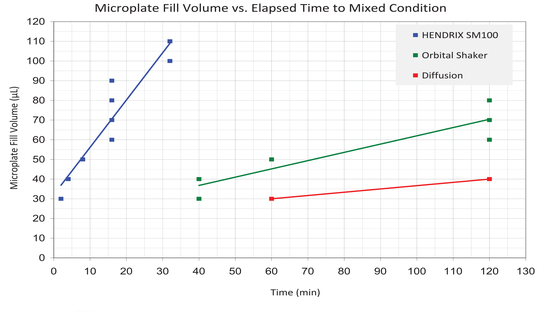

Figure 3. Fill volume versus elapsed time to mixed condition for the three mixing techniques.

Conclusion

Mixing DMSO into water, such as when compounds from a library are plated into screening assays is much more difficult than is commonly known. Incomplete mixing can cause considerable variability, and as screening volumes decrease the problems become more pronounced. This study shows that while mixing by diffusion or aggressive mechanical mixers can be accomplished, it requires considerably longer times than is practical. However, the HENDRIX SM100 can cause rapid and effective mixing in just a few minutes, particularly for smaller assay volumes where traditional mixing is hampered by surface tension effects.

Future Work

Microsonics intends on extending our mixing methodology to other common plate formats and mixing techniques. Please suggest what you would like to see in our next effort!

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