PHOTOMETRIC MEASUREMENT OF MIXING EFFICIENCY USING THE EPPENDORF MIXMATE® PLATE MIXER

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OVERVIEW

- Efficient mixing of solution components is a critical step in the performance of certain assay types. As more life science and pharmaceutical laboratories conduct their assays in 96- and 384-well plate formats, mixing issues within these plates are becoming more and more common. However, these plate formats and their special geometries, in combination with reduced reaction volumes, place very specific demands on efficient mixing. In particular, 384-well plates can present difficult mixing challenges for classical mixers.
- In an effort to find a mixing device that optimally mixed in 384-well microtiter plates in one minute or less, Artel performed an evaluation of the Eppendorf MixMate plate mixer. Instead of relying upon subjective visual observations, Artel has developed a quantitative dual-dye absorbance method to assess the capabilities of mixing devices. In this method, two dye-containing solutions (a sample solution plus a diluent solution) were dispensed into the same wells of a 384-well microtiter plate. Absorbance values of the dyes in each well were photometrically measured before and after mixing. A premixed combination of the two dye-containing solutions was used as a Control to account for any potential non-mixing related artifacts. The relative variation in average absorbance and coefficient of variation (CV) computed over multiple mixing trials provide data regarding the mixing efficiency of the device.

PURPOSE

- Utilize a dual-dye photometric method to evaluate the mixing efficiency of the Eppendorf MixMate with a specific 384-well microtiter plate.
- Determine the speed and time that produces optimal mixing in the 384-well microtiter plate.

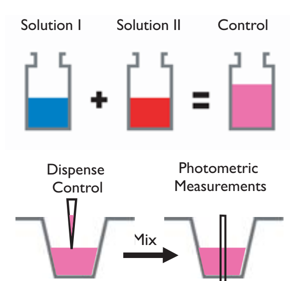
MATERIALS

- ARTEL MVS® Diluent (solution I) and Range C (solution II). Solution I contains a fixed, known concentration of blue dye. Solution II contains the same concentration of blue dye as solution I but also contains a known concentration of red dye.
- Eppendorf MixMate® plate mixer.
- Plate reader to measure absorbance at 520 nm & 730 nm.
- 384-well, flat bottom, non-treated black polystyrene microtiter plates (Corning, #3711). Well geometry is square (with rounded corners).

METHOD

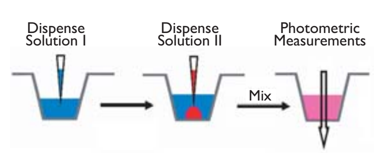
Control

- A set number of columns (or wells) are filled with 55 µL of a premixed Control solution. This Control solution, which is mixed before being dispensed into the test plate, consists of the two dyes in the same concentration as the test solutions. In addition, since the blue dye is at the same concentration in both solutions, it is used as an internal standard.



Sample

- Wells under test are filled with 53 µL of solution I.
- Using a multichannel pipette, 2 µL of solution II is added to the wells containing 53 µL of solution I.
- Remaining empty wells are filled with 55µL of solution I. This replicates the normal weight of the plate during the mixing process.
- The plate is read for initial/premixing absorbance values. This pre-mix read is referred to as Trial 0 in the Figures.



- After the initial reading, the plate is subjected to a predetermined mixing program performed by the Eppendorf MixMate under test. Once the program is complete, the plate is re-read. The process of mixing/reading is continued for a set number of trials.
- For each trial at both wavelengths, the average absorbance, standard deviation, and coefficient of variation (CV) for each test well and control well are computed. This includes the internal standard, which acts as a control in both solutions. Thus the data provides information regarding the efficiency of the mixer and mixing program.
- Due to the fact that the Control solution is already mixed when dispensed into the microtiter plate, the average absorbance and CV should start and remain approximately the same over the multiple mixing trials. The data generated from the Control solution can remove the effects of any potential delivery artifacts (e.g. solution evaporation and splashing). Finally, the reader should note that the absorbance of the control and sample do not need to converge.
- The initial average absorbance and CV data for Test wells filled with solution II will often produce inflated values (although it is possible to observe lower values) compared to post-mix data. This is primarily due to an area of concentrated red dye from solution II in the center of the wells. After mixing, the measured parameters will shift to a new value. As the red dye is mixed and is evenly distributed, the absorbance changes and the CV decreases since CV is a measure of relative dispersion. If the two solutions are optimally mixed, further mixing trials will not produce values with significant variation from the first post mixing results.
- In some cases, mixing parameters are not sufficient to optimally mix the solutions with a single trial. To the eye, the solutions may appear to be completely mixed. However, further mixing trials may provide quantitative evidence that this conclusion is false. As the additional mixing trials are completed, measured parameters will shift or trend towards a relatively stable plateau of values. Therefore, once the values are no longer changing after each mixing step (i.e. the plateau), optimal mixing has occurred.

RESULTS

Increased Mixing Time

- Using the described dual-dye photometric method, the MixMate plate mixer was tested for optimal mixing in 384-well Corning 3711 microtiter plates.
- The setting for this test was the preprogrammed 384 soft key parameters of 15 seconds at 2000 RPM. The MixMate visually appeared to produce mixed solutions after one mixing trial. However, the photometric measurement tests concluded that the solutions were not completely mixed (Figures 1-4).
- As shown in Figures 1-4, the absorbance values reach a plateau after four mixing trials. This indicates that the MixMate did generate optimally mixed solutions after four mixing trials (a total of 60 seconds).

FIGURE 1

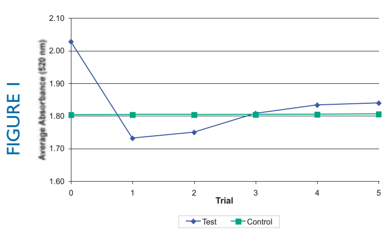


FIGURE 2

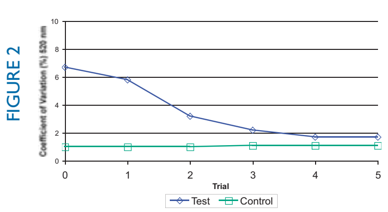


FIGURE 3

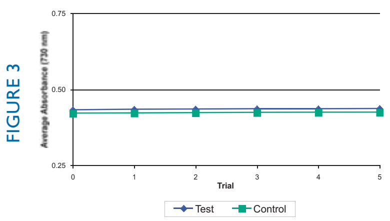
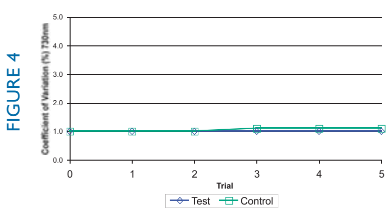


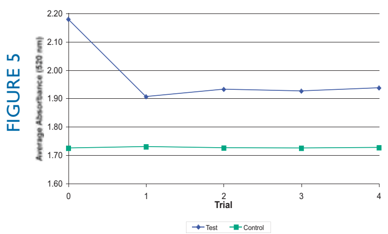
FIGURE 4



Increased Mixing Speed

- A second set of tests was conducted to determine the effects of increasing the mixing speed. The settings for this test were 15 second trials run at 2300 RPM (faster speeds had a tendency to produce splashing of the solutions).
- As shown in Figures 5-8, if the mixing speed is increased to 2300 RPM the plateau level is reached after two mixing trials. These data show that the MixMate did generate a mixed solution after two mixing trials (30 seconds).
- It should be noted that the experimental data in Figures 5-8 were collected using a different type of plate reader than the data in Figures 1-4. Although there is a deviation in the %CV data for the 730 nm measurement (Figure 8), it is not considered to be significant because both the Test and Control exhibit the same pattern. While the exact nature of this deviation is not known, one explanation may be due to a slightly noisier absorbance measurement at the 730 nm wavelength.

FIGURE 5



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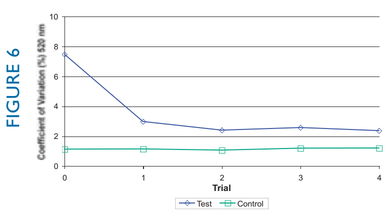

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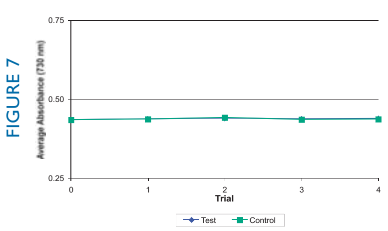
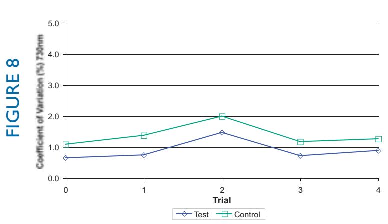


FIGURE 8



CONCLUSIONS

- The dual-dye photometric method discussed in this poster provides a quantitative test to measure, evaluate, and adjust mixing parameters with the Eppendorf MixMate. The method measured the efficiency of the MixMate to optimally mix two similar aqueous solutions, in a specific 384-well microtiter plate. The MixMate successfully achieved mixing of these solutions in this specific plate type, as shown by the data obtained using the dual-dye mixing method.
- In the first test, the dual-dye method was applied as the mixing time (seconds) was increased. The method indicated that at 2000 RPM, the total mixing time should be increased to 60 seconds. This provided a sufficient amount of time for optimal mixing to occur. In the second test, the dual-dye method was applied once again as the mixing speed (RPM) was increased. The method indicated that 2300 RPM was sufficient to not only avoid splashing, but it also resulted in optimal mixing. In addition, at this higher speed, only 30 seconds were required to optimally mix. Under these test conditions, the dual-dye method results exhibit the expected absorbance and CV data indicating optimal mixing of both solutions.
- The results from these experiments show that the MixMate can overcome the "difficult" mixing requirements of the 384-well Corning 3711 microtiter plate and produce optimal mixing in less than one minute.

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