Hudson Robotics, Inc.

AppNotes

DYE ABSORPTION TECHNIQUE FOR VALIDATING THE PRECISION OF THE SOLO AND MICRO10X

The Hudson Solo Plus Pipettor/Dispensor

Introduction

Users frequently ask for a simple and reliable method for determining the precision of their SOLO and Micro10x liquid handlers. The two main approaches involve gravimetric and spectroscopic analysis. The former, which is covered in another AppNote is only appropriate for validating the performance of the single-channel varieties of the SOLO. Spectroscopy is the only feasible method for analyzing multiple channels dispensing simultaneously.

Methodology

The simplest spectroscopic methods involve dispensing a volume of liquid containing a chemical species that produces a reproducible signal at a known frequency. The magnitude of the signal is related, via Beer's law, to the volume of the sample. The precision of the Solo or Micro10x can be determined by filling multiple wells with the same amount of solution and comparing the OD for each well. In this method, we use standard blue food coloring, Brilliant Blue #1, which absorbs strongly at 630nm.

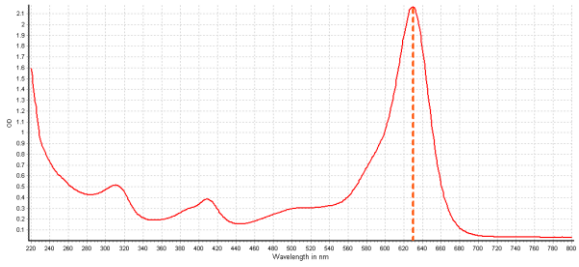

Figure Three: Plot of Optical Density (OD) versus wavelength of Brilliant Blue #1.

Experimental

A standard blue dye solution was prepared by dissolving 3g of standard Brilliant Blue #1 (McCormick) in 900mL of distilled water. At this concentration, a 100uL sample in the well of a 96-well plate absorbs with an OD of 2.1.

A 4-chamber reservoir (Seahorse S-0051) was placed in deck position 3 of the SOLO and one chamber was filled with the blue dye solution. 200uL tips were placed on deck position 1 and a 96-well UV-transparent plate (Corning 3635) was placed on deck position 2. The Solo was equipped with a Gilson 200uL 8-channel pipette.

A SoloSoft procedure was executed that filled the entire 96-well plate with 100uL of blue dye solution. The tips were not changed between

columns, and the tips were pre-wet by first aspirating 100uL and returning it to the reservoir. The z-shift of the aspirate step was chosen so that the tips penetrated the solution in the reservoir by 1-3mm. Using a large reservoir ensures the depth of penetration is the same for each of the 12 transfers.

The full plate was transferred to a BMG LabTech spectraSTAR reader to obtain the absorbance spectra. This instrument allows one to obtain the entire spectrum for each well in less than 2 minutes. A standard monochronometer or filter-based instrument can also be used. In either case, optical densities (OD) are collected for the absorbance at 630nm.

The ODs are tabulated and averages, standard deviations and CVs are calculated for the whole plate (96 values), for each dispense (12 x 8 values and for each channel (8 x 12 values).

Results

Figure Two shows a plot of ODs obtained for each of the twelve 8-channel dispenses described above.

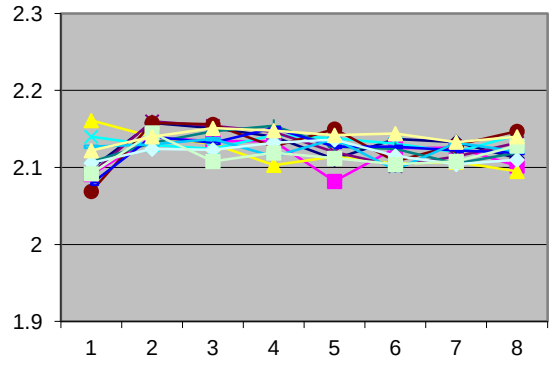

Figure Two: Absorption results from 12 8-channel dispenses of 100uL of blue dye solution

The following table summarizes the data obtained with in this experiment. The first row shows the overall statistics based on all 96 wells. The average OD is 2.13 with a standard deviation of 0.02, which corresponds to a CV of 0.91%. The second row shows the data obtained from each of the 12 8-channel dispenses. Although the average CV is 0.85%, the individual experiments varied from 0.4 to 1.42%. In row three the results are organized by individual channel which showed a similar variation in statistical precision. These results compare with the spreads in figure two, which shows channel 1 has the greatest variation (CV = 1.25%) and channel 7 has the least (CV = 0.54%).

	Avg OD	Std Dev	CV (%) Avg	CV (%) Range
Whole Plate 1x96	2.13	0.02	0.91	0.91
Dispense 12 x 8	2.13	0.02	0.85	0.40 - 1.42
Channel 8 x 12	2.13	0.02	0.77	0.54 - 1.25

We have used the same method to determine the precision of the Micro10x dispenser. Typical results are shown in the following table. The manifold of the Micro10x shows a bit more variation per channel, so the dispense numbers are higher (2.00 – 4.36), whereas, the reproducibility per channel is tighter (1.24 – 2.85).

	Avg OD	Std Dev	CV (%) Avg	CV (%) Range
Whole Plate 1x96	1.8	0.05	2.67	0.91
Dispense 12 x 8	1.8	0.05	2.67	2.00 - 4.36
Channel 8 x 12	1.8	0.03	1.83	1.24 - 2.85

Conclusion

This method represents a simple, fast and accurate method of determining the precision of multi-channel liquid handlers such as the 8-channel Solo and Micro10x.

Hudson Robotics, Inc
973-376-7400
http://www.hudsonrobotics.com
info@hudsonrobotics.com