Hudson Robotics, Inc.

AppNotes

SERIAL DILUTION WITH THE SOLO

The Hudson Solo Pipettor

Introduction

Many biological assays begin with a serial dilution step. This procedure is used in assay development to determine the optimum concentration of various components, and it is the standard method of preparing a range of concentrations of potential drug candidates.

The SOLO is an excellent instrument for carrying out serial dilutions, and excellent results are easily achieved by choosing the appropriate settings in the aspirate and dispense steps within SoloSoft.

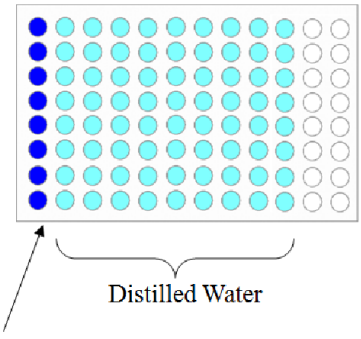
In this AppNote, we describe a simple method to create and evaluate the quality of a serial dilution using blue food coloring and measuring the results with an absorbance reader.

Methodology

In a standard serial dilution, a stock solution of sample is placed into a well in the first column of a plate and the remaining columns of that row are filled with the diluent. A portion of the stock solution is then transferred to column 2 and mixed to form the first dilution. The ratio of the diluent volume to the amount of stock solution transferred determines the degree of the serial dilution. For example, a 2X serial dilution is obtained by transferring 50uL of stock solution to 100uL of diluent. Once thoroughly mixed, 50uL is aspirated from column 2 and added to column 3 to make a solution with one fourth the concentration of the stock. This is repeated as many times as desired.

In this example, we use blue food coloring which absorbs at 630nm. We'll fill 9 rows with dilute water and measure the absorbance at each concentration. We will be running a 3:2 serial dilution to keep the concentration from dropping so low that we can't read many of the dilute samples.

a



b

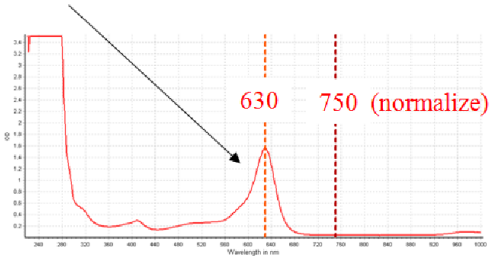


Figure One: (a) Layout of plate for serial dilution **(b)** Absorbance spectrum of blue food coloring

Experimental

Prepare a concentrated solution of the dye by

mixing 400uL of McCormick's blue Food Color & Egg Dye and 10mL distilled water.

Pipette 200uL of the dye solution into all 8 wells of column 1 of a 96-well microplate (we recommend Costar 3635 plates). Fill all the wells in column 2-10 with 120uL distilled water.

Place a box of 100uL Hudson disposable tips on position 1 of the SOLO and place the filled microplate on position 2. Run the SoloSoft procedure described in figure 2. When complete, place the plate in an absorbance reader and collect the OD values for the peaks at 630 and 750nm.

SoloSoft Procedure

The SoloSoft procedure only contains 5 steps, and is summarized in figure 2. Fresh tips are loaded in step 1, and a loop with count 10 is then added, along with the corresponding "end loop" step at the end.

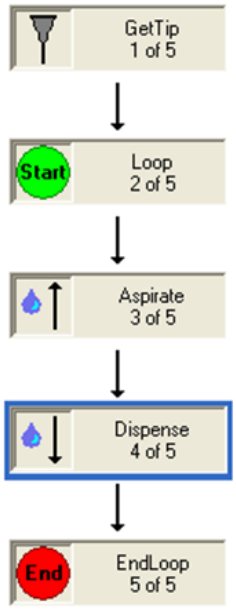


Figure Two: SoloSoft Protocol Steps

The key steps are the aspirate and dispense steps that go inside the loop. Select deck position 2 in both steps and make sure the plate type is correct. Check "empty syringe before aspirate" option in the aspirate step and set the aspirate position low enough to draw all of the liquid (we suggest -11mm). The dispense position for the dispense steps should be set somewhat higher (-9mm) to maximize the effect of mixing, which should be carried out 5 times with a volume of 100uL.

We used 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. Alternatively, a standard monochronometer or filter-based instrument can be used. In either case, collect data for the absorbance at 630nm and 750nm. Subtract the 750nm value from the 630nm to maintain a common baseline and determine the statistics for each column.

Results

The CVs obtained for the various columns are between 1-3%. Depending on the concentration of food coloring, the first two columns will be too high and saturate, or the last two or three columns will be too low to provide meaningful data.

Figure Three shows the relationship between absorbance maxima and concentration. In this run the top two concentrations were saturated, so the optical density peaks at 3.5.

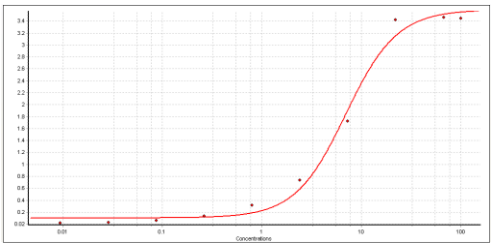


Figure Three: Plot of Absorbance of blue food coloring as a function of concentration

Conclusion

This method represents a simple and inexpensive method of ensuring the settings used in a serial dilution protocol are optimized. It also tests the consistency of each channel in a multi-channel SOLO.

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