

Base Line Instrument Verification for Low Volume Dispensing Precision and Accuracy

BioTek P/N 7773001, Blue Test Dye, is a material called for in many BioTek IQ/OQ/PQ procedures, but it can also be used as an inexpensive, fast, and confident base line indicator of instrument performance at any time. For really fast but flexible verification, you can just use the Combination Test described in step 3. It is assumed that any instrument programming only needs to be done once (not described here), then archived to repeat at any time.

1. Prepare a kit that contains the portable(**) items necessary for the test, and try to use the same detection and weighing instruments each time you run the test.

Materials Required

Precision balance with minimum capacity of 100 g and readability of 0.001 g resolution

Microplate reader capable of reading 630 nm

Orbital or other microplate shaker

BioTek Blue Test Dye P/N 7773001**

384 well clear or black sided clear bottomed microplates**

Variety of Graduated cylinders**

250 mL or appropriate size containers w/lids for making and storing dye solutions**

Deionized water source

For Precision: either the 50 mL reagent reservoirs or 65 mL reagent reservoirs**; carriers for the reagent reservoirs**, plate carrier**, 50 or 200 ul tips** (all provided with the instrument)

For MicroFlow or EL406: 1 µl, 5 µl, and/or 10 µl cassette(s)**

Any other manual pipettes, tips, and reservoirs as desired or found required**

2. Prepare the dye solution for the intended test volume. For example, make the 5 µl dye solution for testing the accuracy and precision of a 5 µl dispense. These instructions make 100 mL, because it is useful to have a stash of each volume on-hand in the test kit.

To Make 100 mL of Test Dye Solution

Test Volume (µl)	Amount Dye (µl)	Amount Dei H2O (mL)	%Dye:Total Volume	Well Dispense Volume Dye (µl)	Well Dispense Volume DeiH2O (µl)	Total Volume in Well (µl)	% Dye to Final Well Volume	Expected OD per Well
2	4000	96	4%	2	23	25	0.32%	1.0 OD +/- 0.2 OD
5	1900	98.1	1.90%	5	20	25	0.38%	1.0 OD +/- 0.2 OD
10	910	99.10	0.91%	10	15	25	0.36%	1.0 OD +/- 0.2 OD
15	690	99.31	0.69%	15	10	25	0.41%	1.0 OD +/- 0.2 OD

Please note: the EL406 syringe drives and Precision are only specified for volumes as low as 5 µl

3. Choose a dispense profile that matches your application. If you are dispensing 5 µl to fluid already in a well, or are dispensing fluid to 5 µl already dispensed to a dry well, mirror the profile in the testing sequence. The Combination Test does both by offering accuracy and precision for multiple dispenses of low volumes in a combination of dry and wet well patterns using only the 10 µl test dye solution.

Dry Well Test Profile Procedure

- A. Tare the microplate
- B. Dispense the test volume to all wells of the 384-well plate
- C. Weigh the plate and record the measurement
- D. Dispense deionized water to the test dye volume in the well to make a final volume of 25 μl in the well. For example, if 5 μl of test dye solution is dispensed to the plate, dispense 20 μl of deionized water to make 25 μl in the well.
- E. Weigh the plate and record the measurement.
- F. Shake the plate approximately 1 minute at medium speed.
- G. Read the plate at 630nm.
- H. Go to section 4, Analyzing Data

Wet Well Test Profile Procedure

- A. Follow all steps as for the Dry Well Test Procedure except swap steps B. and D., dispensing the deionized water first, and the test dye solution second.

Combination Test Profile Procedure: The 10 μl test dye solution is dispensed to the well in three different ways, each dispense combination totaling a final volume of 10 μl of the dye and 15 μl of the diluent.

- A. Tare *three* microplates
- B. In one plate dispense 2 μl of the 10 μl test dye to all wells of the plate.
- C. Weigh the plate and record the measurement
- D. Repeat steps B and C four more times for a total of five 2 μl dispenses to the well.
- E. Alternatively, mix up the 2 μl dispenses with step F. in any combination (some before and some after). Always perform step G. following an individual dispense step.
- F. If step D., dispense 15 μl of deionized water to the well. If step E., dispense the 15 μl deionized water at any point in the five 2 μl dispense steps. Always perform step G. following an individual dispense step.
- G. Weigh the plate and record the measurement.
- H. Shake the plate at least 1 minute on medium speed
- I. Read the plate at 630nm
- J. To the **second** plate dispense 5 μl of the 10 μl test dye to all wells
- K. Weigh the plate and record the measurement
- L. Dispense 15 μl of deionized water to the plate
- M. Weigh the plate again, record the measurement
- N. Dispense 5 μl of the 10 μl test dye to the plate
- O. Weigh the plate again, record the measurement
- P. Repeat steps H. and I.
- Q. To the **third** plate dispense 5 μl of deionized water to all wells of the plate
- R. Weigh the plate and record the measurement
- S. Dispense 10 μl of 10 μl test dye to each well
- T. Weigh the plate and record the measurement
- U. Dispense 10 μl of deionized water to each well
- V. Weigh the plate and record the measurement
- W. Repeat steps H. and I.

4. Analyze the data. Here are the calculations I perform at the end of the test. If any of the data is questionable I repeat the test first before undertaking any other troubleshooting maneuvers. I also make sure my procedures are correct, and the instrument is setup correctly. If any of the data appears dramatically outside the instrument specifications, and it is repeatable, then I generally undertake more extensive troubleshooting – not included here!

How to Calculate Results

- A. I use Gen5 software to run the microplate reader, so I create a map that has one sample with 384 replicates. This gives me an immediate mean and CV% calculation for all wells on the plate under the Statistics tab at the end of the run. I then import the data to Excel to do the rest of my analysis.
- B. The CV% for the plate should reflect instrument specifications for the test volume. There is some leeway here, though, because the tests are not exact replications of those used to determine instrument specification ranges. When I see a CV up around 10, a flag goes up to look closer at the data, and sometimes I repeat the test just to get another data set. The mean should be 1.0 OD +/- 0.2 OD (this indicates the dyes were prepared correctly).
- C. To check consistency of the dispense volume across the plate, and find any notable patterns (e.g. drift across the plate, or spiking patterns) I calculate the mean and CV% for each column and row individually.
- D. I calculate the delta and ratio of the Min and Max OD of the plate to confirm tight dispense volume uniformity across the plate.
- E. Steps A-D are my precision data.
- F. I calculate % accuracy on the plate as follows:

Description	Formula	Result
1. Calculate actual volume by transforming g weight to μl per well	$\text{g weight from recorded weight measurements} / \text{number of wells} (384) * 1000$	This should reflect expected volumes for each dispense
2. Transform μl / well to % accuracy*	$\text{actual volume} / \text{expected volume} * 100$	This should reflect the instrument specification for dispense % accuracy
3. Calculate Delta and Ratio if desired	Ratio = Expected volume/actual volume Delta = Absolute Value (Expected volume – actual volume)	Ratio of 1 +/- 0.02 is ideal** Delta of 0 +/- 0.2 is ideal

**The % accuracy calculation indicates the total amount of liquid in the plate expressed as a per well volume, it doesn't indicate how evenly the liquid is dispersed from well to well, that is what the precision data is for.*

*** Generally, for volumes this low, the ratio of the fluids expected in the well is more important than the final volume in the well. For example, if you are expecting 5 μl on the first dispense, 15 μl on the second dispense, and 5 μl on the third dispense, the ratios of 3 (5:15) and 1(5:5) are a better indication of repeatable and successful instrument performance than a 96.78% accuracy on an actual final well volume of 24.195 μl out of an expected 25 μl .*

5. Keep a log of your results so that the next time you have reference values. This makes it much quicker to do the data analysis and understand how your instrumentation performs over time. This can also help determine when regularly scheduled maintenance routines should occur.