Technical Note: 08003

Thermo Scientific PocketTips® Nanoliter Accuracy and Precision Testing Procedure

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The following procedure outlines the suggested materials and process for evaluating the accuracy and precision of Thermo Scientific PocketTips by performing a titration curve and comparing the average readings of the PocketTips' dispense to the curve.

Introduction

PocketTips, nanoliter dispensing tips, feature an internally molded capillary pocket for transferring 50 nl to 500 nl of sample volume using a laboratory's current automated liquid handling instrumentation. By enabling low volume transfers directly to an assay plate, PocketTips eliminate many timely intermediate dilution pipetting steps, resulting in a reduction in reagent, labware and sample costs, and soft in-tip mixing improves data quality. PocketTips are designed to fit automated liquid handling instrumentation like standard format automation tips, requiring minimal instrument modifications.

Consistent dispensing accuracy and precision is tightly controlled during the molding process of the internal pocket and is independent of the pipetting instrument. A coefficient of variance of less than 6% can be achieved for any pocket size.

Oregon Green® powder is a 2' 7'-difluorofluorescein dye that is soluble in DMSO. Unlike other fluorescein dyes that can bleach via fluorescent light and have proven difficult for generating accuracy of nanoliter volumes data, Oregon Green powder is very stable and does not easily bleach or drift, resulting in accurate test data.

Materials

1. Oregon Green Powder, dissolved in 100% DMSO
2. DMSO Concentration, Approximately 100 µM
3. PBS Buffer or DI Ph Neutral
4. Polystyrene Assay Microplate, Black, with 25-50 µl PBS or DI in Plate
5. Calibrated Handheld Pipette
6. Calibrated Fluorescence Microplate Reader
7. Microsoft® Excel®

Methods

Perform a Titration Curve

1. Add 95 µl of DI water to well A1 of assay plate
2. Add 50 µl of DI water to wells B1 through H1 of assay plate
3. Using the calibrated handheld pipette, add 5 µl of Oregon Green DMSO (50-100 µM stock) into well A1 of assay plate
4. Discard and replace handheld pipette tip
5. Using handheld pipette, mix well A1 with 50 µl volume for 15 iterations
6. Transfer 50 µl from well A1 to well B1 and mix well with 50 µl volume for 15 iterations; repeat through well H1
7. In well H1, mix well and discard the remaining 50 µl
8. Using a calibrated fluorescence microplate reader read wells A1 through H1 and plot the resulting curve (Please note: the reader data in A1 will correspond to 2.5 µl, B1 will correspond to 1.25 µl, etc.)
9. Use the Microsoft Excel function to determine the R-square, slope and intercept of the curve values (see Figure 1)
10. Select the reader data that produces an R-square of at least 0.999 (For example, select reader data C1-H1; A1 and B1 data may be saturated and need to be discarded)

Procedure Performed with PocketTips

1. Fill a second assay plate with 50 µl of DI water and transfer DMSO into the plate using PocketTips
2. The PocketTip volume (50 nl, 100 nl or 250 nl) should match the titration curve.

Results

Testing with PocketTips should yield <6% CV's across a full plate with accuracy volumes within +/- 10% of the target.

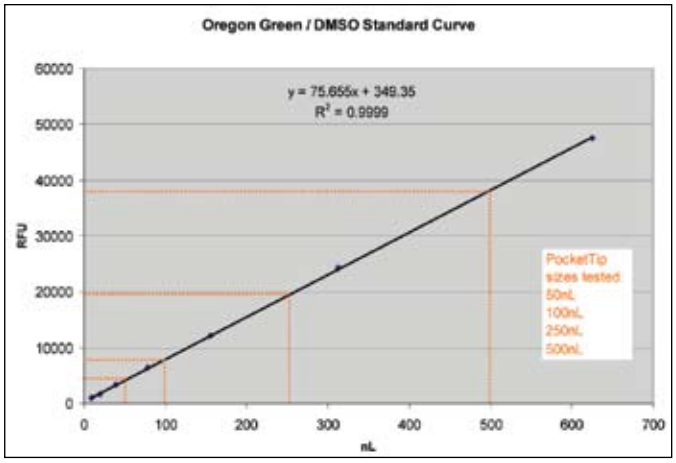

Figure 1:
Resulting Standard Curve

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