Technical Data for the Quad-Z 215 (fixed probes), Dispensing Small Volumes of DMSO

Application Note 200

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Introduction

As today's assays require smaller and smaller volumes, researchers are requiring accuracy and CV data to be available for an increasing number of solvents. This study determined the accuracy and CVs for the Quad-Z 215 (fixed probes) for low-volume dispensing of DMSO. The volumes (1, 3, 5, 7, and 10 µL) of DMSO were transferred from a 96-well format into an empty, flat-bottomed, 96-well microplate.

Materials & Methods

Chemicals and Reagents

Methylene blue

DMSO

NANOpure® water

Instruments and Accessories

Gilson Quad-Z 215 Liquid Handler, equipped with: 175-mm Z-arm with four independent shafts and non-septum piercing, constricted-tip probe (269 x 1.3 x 0.8 mm)

Gilson 444 QuadDilutor, equipped with: four independent syringe drives and 250-µL syringes

Gilson 735 Sampler Software

Gilson Code 201 and Code 201H Racks

Molecular Devices MAXline Vmax® Kinetic Plate Reader

Costar® clear, flat-bottomed, 96-well microplates

Description of the Procedure

The volumetric testing was done using methylene blue solutions. To create a 2.5 mg/mL stock solution, 250 mg of methylene blue was dispensed into a 100-mL volumetric flask. 10 mL of DMSO was added to this solution and brought to volume with NANOpure water. To create a 50 µg/mL stock solution, 1 mL of the 2.5 mg/mL stock solution was added to a 50-mL volumetric flask and brought to volume with DMSO.

A standard curve was manually generated using these two stock solutions. The resultant concentrations for the standards (µg/mL) 50, 15, 10, 7, 5, 3, 2, 1, 0.75, and 0.50 were generated, and 200 uL of each solution was added to a clear, flat-bottomed Costar microplate. The additions of the standards or blank

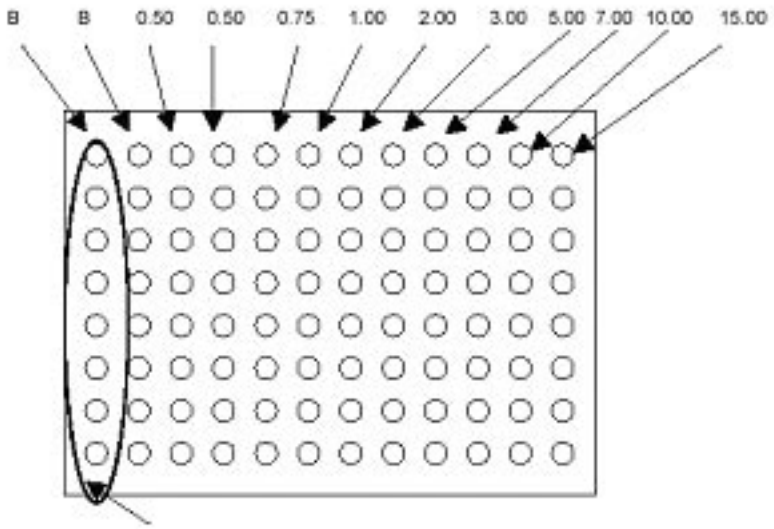

Figure 1: Standard Curve Plate

The concentration (µg/mL) of the standards are noted above the diagram; B = blank (water).

To ensure greater low-volume accuracy, each volume used a 6-µL air gap and an extra volume (50 µL) of DMSO. The dispense flow rate was 1.0 mL/min. A probe height of –1.50 mm was used for the result zone plate to accommodate a touching-off of the droplet during the dispense task. A generic shallow, 96-well microplate was used as the source plate containing the DMSO/methylene blue solution to be dispensed into the empty, clear, shallow-well Costar microplate.

The generic source plate was placed on a Gilson Code 201H Rack to allow placement of a single sheet of filter paper on top of the plate. This allowed the outside of the probes to be wiped while retracting from the source plate. The Costar destination plate was placed on a Gilson Code 201 Rack. To determine the volumetric accuracy of the Quad-Z 215, a working solution of methylene blue in DMSO was used. The working solution consisted of a 1:10 dilution of 2.5 µg/mL methylene blue solution in DMSO. The volume was transferred from the source plate to the Costar plate, after which the droplet was brought to volume with 200 µL of NANOpure water. Therefore, for a 1-µL droplet, the final concentration would be 1.25 µg/mL. The plates were read on a Molecular Devices plate reader at the following settings:

- Auto mix: once
- Wavelength: 650 nm

Results

Volume Absolute (µL)	Average Volume Observed (µL)	CV (%)	Accuracy (%)	STD (%)
1.0	1.04	2.12	104	0.01
3.0	2.95	1.61	98	0.01
5.0	4.89	1.39	98	0.02
7.0	6.9	1.18	99	0.03
10.0	10.13	1.1	101	0.03

Table 1: Average of the Four Probes Across a 96-well Microplate

The actual data for each probe at each volume dispensed is available upon request. (For additional information, please contact the Gilson, Inc. marketing department.)

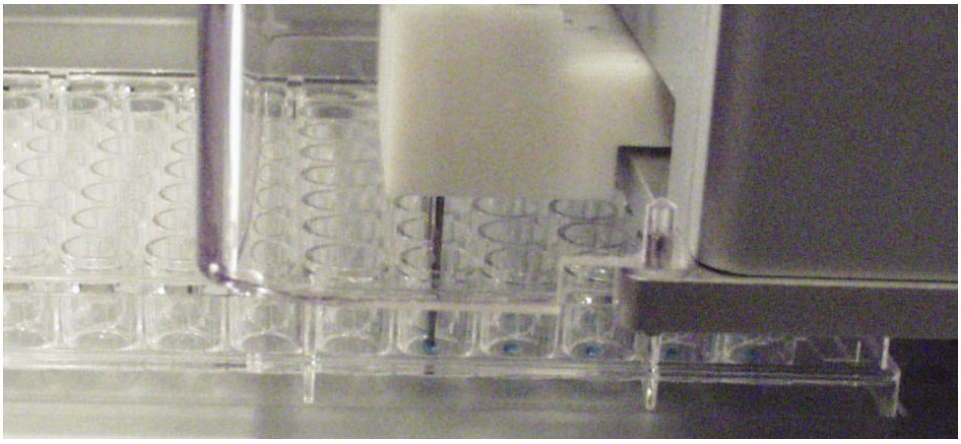

Photo 1: Side View of the Quad-Z 215 Dispensing 1-µL Droplets of DMSO into an Empty Microplate

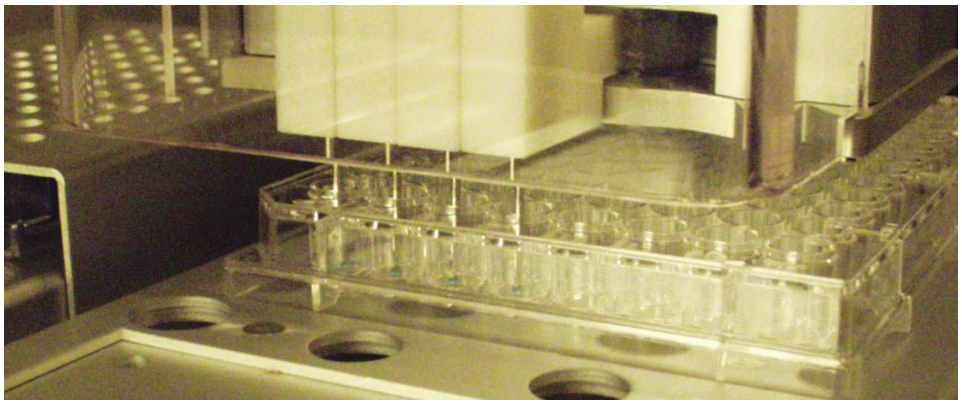

Photo 2: Front View of the Quad-Z 215 Dispensing 1-µL Droplets of DMSO into an Empty Microplate

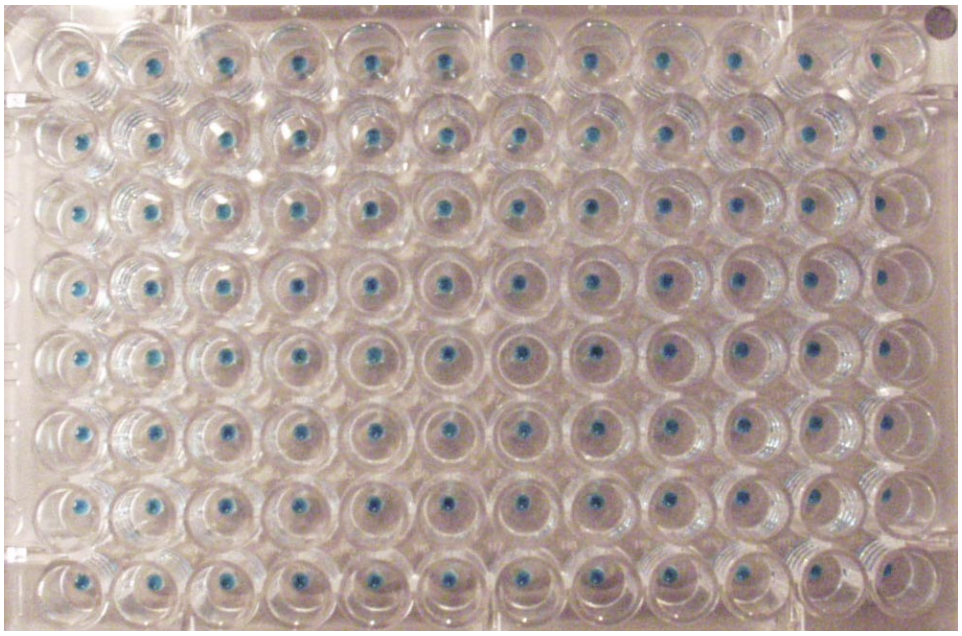

Photo 3: Overhead View of a 96-well Microplate after Dispensing 1-µL of DMSO per Well

The aliquot task in 735 Sampler Software was used to dispense the droplets across the entire plate. The parameters used for the aliquot task are as follows:

Volume Transferred: 1, 3, 5, 7, and 10 µL

Extra Volume: 50 µL

Air Gap: 6 µL

Aspiration Flow Rate: 0.5 mL/min.

Dispense Flow Rate: 1.0 mL/min.

Inside Rinse: 1000 µL

Outside Rinse: 1000 µL

It took 2.45 minutes to complete the entire procedure for a 96-well microplate. To ensure optimal results, an initial rinse prior to the transfer was implemented. The rinse required 1.0 minute to complete, therefore, 1.45 minutes is required for the transfer of the DMSO. Hence, the addition of DMSO to eight plates could be accomplished in less than 15 minutes.

Summary of Results

As shown from the results of this testing, the Quad-Z 215 (fixed probes) is capable of transferring low volumes of DMSO with CVs for 1 µL in the 2.2% range and 1.6 –1.1% for the 3–10 µL volume transfer into empty, flat-bottomed, 96-well microplates.

When dispensing into an empty, flat-bottomed plate, it is imperative that the probes used in this study (flat, constricted tip) be used. A different probe—the micro beveled-tip probe, which would seem to be the logical choice—facilitates the wicking of the DMSO on the outside of probe instead of dispensing the DMSO into the well. Also, the beveled nature of the micro probe doesn't produce the desired touch-off method results required for the low-volume transfer of DMSO. To produce optimal results, this study was completed by configuring the Z-arm of the Quad-Z 215 at a height of 125 mm. This allows the probe to be as close as possible to the plates, decreasing travel length and optimizing the speed and accuracy of the Quad-Z 215.

Conclusion

The ability to dispense low volumes of volatile, hydroscopic, or viscous solvents has become even more important in today's research world with the advent of HTS. Solubilizing synthesized libraries often requires the use of DMSO. Thus, being able to dispense low volumes with precision and accuracy is imperative for concentration determination and assay development. The use of a small amount of solvent can usually be tolerated within a given biological assay. It is crucial that the volume of the solvent remain consistent throughout the dispensing onto the microplate. This allows the results, and, hence, the biological activity, to be correlated to the compounds being evaluated and not the solvent being used.

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