

Rapid Generation of Assay-Ready Serial Dilution Plates using Two-Stage Low Volume Pipetting

Joby Jenkins, Rob Lewis, Tristan Cope, Wayne Bowen
TTP LabTech Ltd, Melbourn Science Park, Melbourn, Hertfordshire, SG8 6EE, UK

Abstract

Profiling the concentration-related effects of test compounds against therapeutics targets requires accurate dilution of stock solutions. In screening laboratories, compounds are routinely diluted from a single stock solution in a stepwise, serial manner across microplates using constant volumes. Recently, a two-stage method of generating serial dilutions has become popular. Stage 1 involves the preparation of multiple stock solutions differing 30-100-fold in concentration. In Stage 2 different nanolitre volumes of stocks are transferred into a second assay microplate to create the 'assay-ready' dose-response curve.

Mosquito® is a low volume liquid handling instrument combining the advantages of a disposable tip system with those of a positive displacement pipette. Mosquito®, in conjunction with the newly developed bulk dispenser module, is fully capable of performing two-stage compound dilution in 384-well microplates using an integrated pipetting protocol. Alternatively, Mosquito® can prepare the multiple stock solutions in Stage 1 for subsequent dilution by acoustic technology. We demonstrate that such procedures can set up assay plates for as many as 370 12-point, six-log, dose-response curves each hour.

Conclusions

- Two-stage dilution on a single platform eliminates integration and provides rapid production of assay-ready plates.
- The direct dilution method eliminates the compounding errors seen with serial dilution.
- Stock dilution in DMSO and Direct Pipetting into assay plates stabilises compound solubility down the concentration series.
- By directly dispensing into assay plates containing cells, dose-response curves can be generated without serial dilution in an intermediate plate.
- A mosquito equipped with a bulk dispenser module can provide assay-ready dose-response plates that satisfy most demands of both biochemical and cell-based screening.

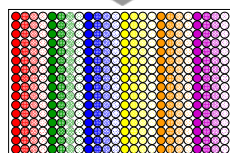
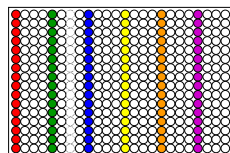
1 mosquito Instrument with Bulk Dispenser Module



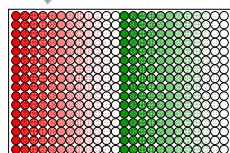
mosquito® is a low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination. mosquito is capable of pipetting volumes from 1.2 µL down to 50 nL with no washing.

2 Two-Stage Approach to Generating Dose-Response Curves

Stage 1 – 96 compound stock solutions in a 384-well source plate are serially diluted (1.5 log) to provide 4 stocks, e.g. 10 mM, 0.316 mM, 10 µM and 316 nM. Diluent is added using the bulk dispenser module.



x3



Stage 2 – Aliquots of each stock (500, 158 and 50 nL) are transferred from the source plate to create 11-point half-log dilution series + blank in three 384-well assay-ready dose-response curve plates, each containing 32 compounds.

3 Stage 1 – Preparation of Step Dilutions



384-well source plate containing 4 step dilutions of 96 compounds

The bulk dispenser module significantly expands the liquid handling capability of mosquito by enabling pipetting protocols combining nanolitre and microlitre volumes. The bulk dispenser module allows high volume ($\geq 10 \mu\text{L}$), non-contact dispensing from 8 independent channels into plates located on the mosquito deck. Low and high volume pipetting steps can be carried out within the same protocol through a common, and simple, user interface. Both components are fully compatible with aqueous and DMSO.

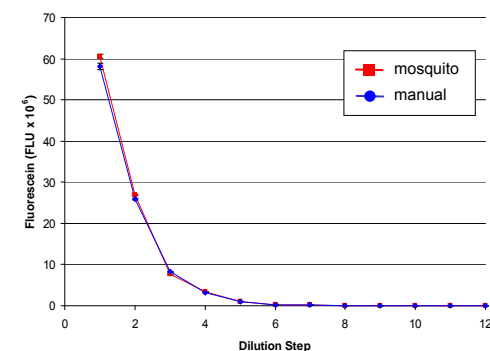
When preparing step dilutions, stock transfer is performed using low volume micropipettes and diluent added using the bulk dispenser module. In the scenario describe here, 96 compounds arrayed in a source 384-well plate are each step diluted 3 times.

- 35 µL of 96 compounds at top stock concentration in 100% DMSO are placed in columns 1, 5, 9, 13, 17 & 21.
- mosquito is used to transfer 1,108 nL of all compounds into the adjacent column.
- The bulk dispenser module adds 34 µL of 100% DMSO to these columns, causing instantaneous turbulent mixing and hence diluting the compound 31.6 fold (1.5 log units).
- The process is repeated twice producing four stocks diluted in 100% DMSO to act as a source plate for Direct Dilution in Stage 2.
- This process takes ~ 4 minutes to complete.

4 Stage 2 – Direct Pipetting Method for Creation of Dose-Response Curves

- mosquito is used to transfer 500 nL, 158 nL & 50 nL of the highest compound stock into columns 1 to 3 in the 384-well assay plate.
- This is repeated for the 3 other stocks, creating up to a 12 point, half-log dose-response curve. The last dilution may be omitted in favour of a DMSO solvent blank resulting in an 11-point dose-response curve spanning almost 6 log units.
- The process is repeated for the next set of 16 compounds in the other half of the assay plate.
- DMSO is added to make up all wells to 500 nL 100% DMSO
- This process takes ~ 3.5 minutes to complete and is repeated 3 times to complete the dilution of all 96 compounds into three 384-well assay-ready plates.

5 Accuracy of Direct Pipetting Dilution Method



The mosquito direct dilution method eliminates the compounding of serial dilution errors. This can result in improved repeatability and significantly reduced errors over traditional automated serial dilutions, where mixing efficiency has a dramatic effect. In tests all column CV's were < 8%. Data shown are for dilution of fluorescein.

6 Direct Pipetting Dilution in Microplates Containing Live Cell Cultures

The use of adherent cell cultures is prevalent in drug screening and often requires addition of test compounds after plating precluding the use of assay-ready plates. Direct pipetting dilution overcomes this problem and furthermore does not require removal of the growth medium. As an example, HeLa cells were added to a 384-well plate and allowed to adhere overnight before treatment with vinblastine (G2/M blocker) for 22 h. Dilution method: columns 1–12 – mosquito direct method; columns 13–24 – manual serial dilutions. For counting, cells were ethanol-fixed, labelled with propidium iodide (10 µM) and analysed on an Acumen®X3 microplate cytometer (TTP LabTech).

