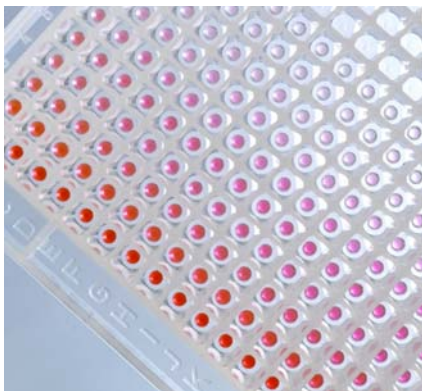


Serial Dilutions

mosquito[®] Application Note

Background

Dilution is an integral step in the pharmacological profiling of compounds, in particular, to determine their concentration-related effects against therapeutics targets. Routinely, serial dilutions are generated in a series of tubes or adjacent wells in microplates requiring a secondary step of transferring the compound dilutions into the assay plate.



mosquito[®] facilitates the miniaturisation of assays through precise serial dilutions on a microlitre scale. If volumes are restricted to less than 1 μ L, the dilution plate may be used as the assay plate.

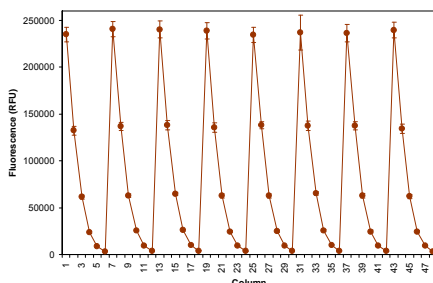
mosquito[®] is also able to sample nanolitre quantities direct from cherry-picked picotubes in 384 racks (such as supplied by REMP, TAP and RTS storage systems).

Application

mosquito's micropipettes are arranged in a column of 8 or 16 tips. The pipettes use positive displacement and direct contact, allowing them to aspirate, dispense and even mix sub-microlitre volumes. This enables mosquito to automate assay-ready serial dilutions in 96 and 384 well plates (see methods given on next page).

The most critical aspect of the serial dilution process is the mixing step. It is important to aspirate close to the bottom of the well and dispense near the top of the liquid level as this enhances the mixing process. Improper mixing results in poor CVs and incorrect EC50 or IC50 determinations.

mosquito[®] is also capable of performing serial dilutions in 1536 plates using nanolitre volumes. Hits selected for confirmation were serial diluted (half-log) in a 1536-well plate using a standard 16-tip mosquito and the resultant fluorescence quantified using an Acumen[®] eX3 microplate cytometer (TTP LabTech). The maximum CV for any series of terminal dilutions was 8% (n = 32) indicating the high precision and accuracy of pipetting, as well as good consistency of mixing. Serial dilutions can be transferred into the final assay plate using a pin-tool.



mosquito[®] Features

Zero cross-contamination

Volume range of 50nL – 1,200nL

Negligible dead volumes reduce sample wastage

Addresses 96, 384 and 1536 plate formats

CVs of <8% at 50nL and <4% at 100nl

Accuracy within +/-5% across the volume range

Easy integration with other robotics



Serial dilutions

mosquito® Application Note

There are two methods of performing serial dilutions using mosquito®. Both allow accurate dilution series to be set up in a few minutes using minimal amounts of compound. Both have been proven in HTS environments with biochemical assays.

Method 1: miniaturised traditional method

For a 500nL total volume 7 point half log serial dilution:

- Add 342nL of buffer (typically 100% DMSO solution) to columns 2 to 7 of the destination plate
- Add 658nL of stock solution to column 1 of the destination plate using a clean set of tips
- Transfer 158nL of the stock solution from column 1 to column 2, mixing on dispense.
- If stock compound is 'sticky', tips can be changed
- Transfer 158nL from column 2 to column 3, mixing on aspirate and dispense
- Repeat for all columns in series
- Remove 158nL from column 7 and transfer it to a waste plate to give a final volume of 500nL in all columns.

Method 2: dynamic range method

For a 1uL total volume 8 point 1 in 3 serial dilution:

- Transfer 667nL of buffer (typically 100% DMSO solution) to columns 2 and 6, 889nL to columns 3 and 7, and 963nL to columns 4 and 8
- From a 30mM stock solution transfer 1000nL to column 1
- Using the same tips, multi-dispense 333nL of this solution to column 2, 111nL to column 3 and 37nL to column 4.
- Exchange tips and transfer 1000nL of a 0.3mM solution of the same stock to column 5.
- Using the same tips multi-dispense 333nL of this solution to column 6, 111nL to column 7 and 37nL to column 8

