

# Use of Nanolitre Pipetting in Hit-to-Lead Optimisation

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## Abstract

Assay-ready plates are becoming key to maximizing throughput for many HTS departments. These plates contain test compounds prepared from library stocks ahead of time at the correct concentrations. DMSO is routinely used for compound dissolution, but its presence at concentrations above 1% can markedly affect assay performance. Thus, accurate nanolitre quantities of DMSO-based stock solution are required to keep concentrations low when preparing assay-ready plates. Liquid handlers using disposable tips, such as TTP LabTech's mosquito®, eliminate cross-contamination. Non-disposable pipetting heads can offer high throughputs, but require very stringent wash steps to avoid problems when transferring some compounds.

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. The low volume liquid handling capability of a mosquito combines the advantages of a disposable tip system with those of a positive displacement pipette.

The mosquito has been used to perform low volume serial dilutions in sitting drops located on the bottom of 96 and 384 well microplates. Typically the volumes were restricted to 1-2 microlitres which permitted use of the dilution plate as the assay plate. Results for a high content assay are presented. Use of intra-well dilution can reduce compound usage and consumable costs, as well as increasing assay throughput. In addition, the same pipettor can be applied to high density 1,536 well plates where higher throughputs are required.

Here, we detail the use of nanolitre pipetting in assay-ready plate preparation and its additional use for serial dilution in high density microplates.

## 1 mosquito instrument



mosquito is a low volume liquid handling instrument combining a low cost disposable tip system with a positive displacement pipette. mosquito is capable of pipetting volumes from 1.2  $\mu$ L down to 50 nL with no washing required.

## 2 Accuracy of drop placement

mosquito's X, Y and Z axes are accurately driven by stepper motors with a resolution of <0.05mm. This, along with the tightly toleranced and relatively short pipette tips, means that drops can be placed with a high degree of accuracy in the centre of wells of any SBS plate, up to and including the high density 1536 format.

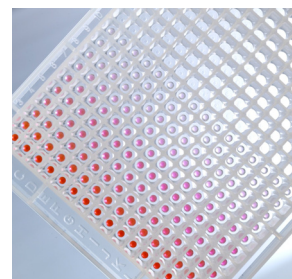
Such accuracy also means that smaller drops can be placed directly on one another, without the worry of drops not coinciding or being distorted by the well walls.

Mosquito's accurate movement and disposable pipettes offer the following advantages:

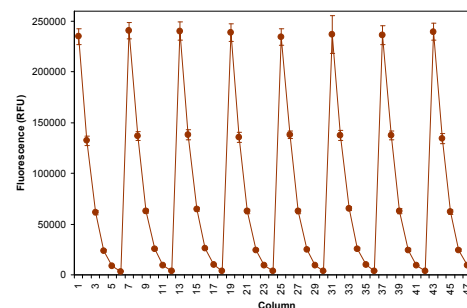
- 50nL to 1,200nL aspirate and dispense range
- positive displacement pipetting handles liquids of varying viscosities accurately without recalibration
- disposable pipettes guarantee zero cross-contamination
- excellent repeatability and accuracy. Mosquito offers CVs of <8% at 50nL and <4% at 100nL across a 384 well plate; accuracy is within +/-5% throughout the volume range
- negligible dead volumes reduce sample wastage

## 3 Automated preparation of assay-ready plates containing serial dilutions

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. This enables mosquito to automate serial dilutions in 96 and 384 well plates, and the transfer of accurate nanolitre quantities into the final assay-ready plate.

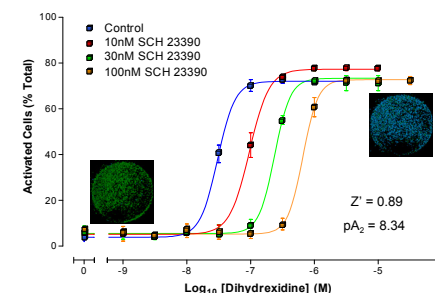


To assess mosquito's suitability for performing serial dilutions in 1536 plates using nanolitre volumes, hits selected for confirmation were serially diluted (half-log) in a 1536-well plate using a standard 16-tip mosquito and the resultant fluorescence quantified using an Acumen Explorer® microplate 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-ready plate using a pin-tool.

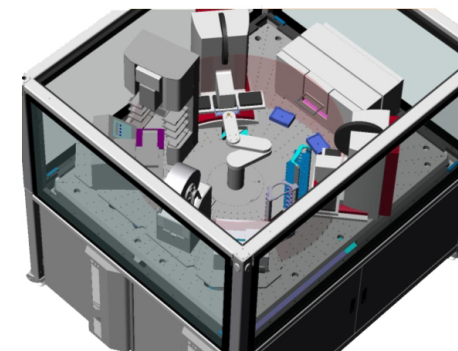


## 4 GPCR screening: $\beta$ -lactamase reporter gene analysis

Assay-ready 96-well plates were prepared containing both serially-diluted dihydroxidine agonist and a fixed concentration of SCH 23390 antagonist. D1 CRE-bla CHO-K1 cells (Invitrogen) were added and treated according to SOP. High content analysis was performed on an Acumen Explorer fluorescence microplate cytometer using 405nm laser excitation.



## 5 A fully automated hit-to-lead screening system



TTP LabTech and Velocity 11 have designed a fully automated high content system incorporating mosquito to perform primary and secondary screens on a compound library. A single system could screen a library of up to 400,000 compounds for hits and then validate those hits in less than 1 week.

