

# A Solution for Serial Dilution of Compounds in 1536-Well Microplates

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## ABSTRACT

High-density screening platforms are an integral part of any successful high-throughput screening facility. The reduced assay volumes associated with increasing well density lowers reagent costs, accelerates assay throughput, and can improve assay quality and reproducibility by minimizing assay duration. Kalypsys' suite of uHTS robotic screening technologies has enabled unprecedented levels of efficiency and economy in a host of screening operations. Based around 1536-well microplates and now in its third generation, the Kalypsys Systems uHTS System has a throughput of over 1 million assay wells per day, and an on-line storage capacity of over 2.2 million compounds. The focus on 1536-well miniaturization has created a need for liquid handlers that are capable of accurately and reliably transferring and diluting small volumes of liquid in 1536-well plates. Kalypsys has identified the TTP LabTech mosquito® as a practical and cost-effective solution for 1536-well low volume serial dilution.

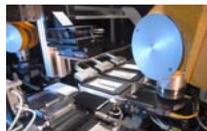
## INTRODUCTION

The mosquito® is an innovative nanoliter pipettor that combines the liquid transfer capability of a fixed head dispenser with the convenience and zero cross-contamination potential of disposable tips. The mosquito® is capable of pipetting volumes from 1.2 µL down to 50 nL with no washing required.

To increase efficiency, Kalypsys stores compounds for screening and hit picking in 1536-well format. In order to save valuable time and resources, as well as to make informed decisions early on in the discovery process, Kalypsys performs serial dilutions on all primary hits and utilizes its proprietary uHTS Screening platform to perform simultaneous confirmation, specificity, cytotoxicity, and P450 testing on these primary hits in 1536-well format. Here we demonstrate the application of the mosquito® for serial dilution of compounds in 1536-well microplates for profiling in biochemical and cellular assays.

## Kalypsys Systems - Integrated Screening System

The Kalypsys system has the capability of screening well over 1 million wells per day. Virtually all biochemical and cell-based primary and follow-up screens are done in 1536-well plates.



All Hit picking is done in 1536 format utilizing Kalypsys Systems proprietary Automated Hit Picker, picking on average over 2500 wells per 24 hours.

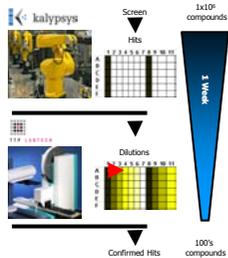
## TTP LabTech's mosquito® - Compact Nanoliter Liquid Handling Solution



- mosquito® is TTP LabTech's low volume liquid handling instrument combining the performance of fixed tip dispensers with the convenience of disposable pipette tips.
- mosquito® is capable of pipetting volumes from 1.2 µL down to 50 nL.
- The ability of the mosquito® to perform serial dilutions was optimized at both the TTP LabTech and Kalypsys facilities.
- mosquito's® small size and simple interface make it an appealing solution.

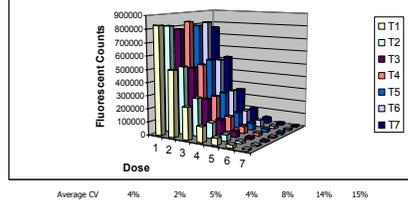
## Kalypsys Screening to Leads Process

- Primary screens are carried out in 1536-well plates.
- Data is analyzed and a hit list is sent to the Kalypsys Systems Hit-picker.
- All libraries on the hit-picker are stored in 1536-well plates with 13 µL of compound in each well.
- Plates containing hits are presented to a liquid handler where 3 µL of compound are transferred into a new plate. (This will make up the first dose of each dilution.)
- For a 7-point serial dilution, this process can accommodate 192 compounds per 1536-well plate plus controls.
- 2.05 µL of DMSO are added to the remaining empty wells of the 1536-well plate.
- The mosquito® then prepares a 7-point half-log serial dilution by transferring 950 nL from column 1 to column 2.
- The well is mixed thoroughly 10 times and the process continues until all 7 doses are complete.
- mosquito® tips are rapidly exchanged before the next set of serial dilutions are prepared.
- Simultaneous confirmation, specificity, cytotoxicity, and P450 assays are performed using Kalypsys Systems uHTS Screening System.



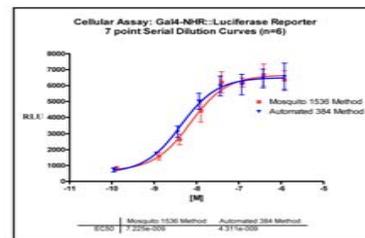
The most critical aspect of the serial dilution process is the mixing step. It is important to aspirate close to the bottom of the well and dispense near the top of the liquid level; this enhances the mixing process. Improper mixing results in poor CV's and incorrect EC50 or IC50 determinations.

## Half-Log Serial Dilutions of FITC in DMSO in a 1536-Well Plate



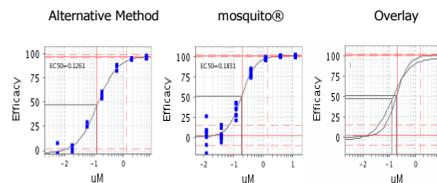
FITC was serially diluted using the mosquito® and the previously described procedure. The experiment shown was done in replicate of 7 dilutions. FITC was diluted in 0.1M TRIS-HCL pH 8 at half log intervals over 7 points starting at 100 nM finishing at 10.6 nM.

## Cell Based Assay Comparison of 1536-well Serial Dilution Using the mosquito® vs. Standard 384-well Serial Dilution



A Luciferase reporter gene assay was used to measure the EC50 of a GAL4-NHR (nuclear hormone receptor) with a control compound. The graph represents 6 replicates of a 7 point dose response across one plate. The published EC50 of the reference compound is 2 nM.

## ELISA - Comparison of mosquito® 1536-Well Serial Dilution vs. a Standard 384-Well Serial Dilution

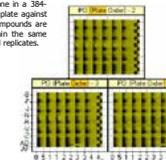


An ELISA was run for a transcription factor complex to compare historic results for a lead compound serially diluted in a 384-well plate vs. the same compound diluted in a 1536-well plate using the mosquito®. Minimal change in the EC50 was observed.

## Fluor de Lys Biochemical Assay (Biomol): mosquito® Dilution in 1536-Well vs. Manual Dilution in 384-Well

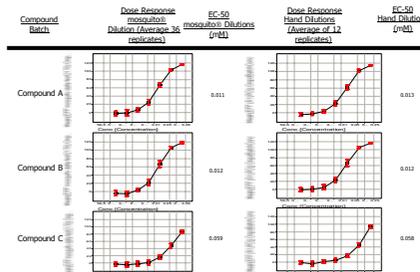
Fluor de Lys, Fluorogenic Deacetylase Substrate, is used to detect HDACs (histone deacetylases). These enzymes deacetylate the substrate allowing it to accumulate inside the cells. These compounds will deacetylate the substrate and accumulate inside the cells. Developer is added to lysed cells containing the sensitized substrate producing a fluorophore. Here we display a visual representation comparing a manual dilution of 15 compounds done in a 384-well plate then transferred in quadruplicate blocks to a 1536-well plate against the mosquito® dilution done directly in a 1536-well plate. The compounds are repeated in 12 replicates. For example, Rows A and B contain the same compound serially diluted in 12 replicates. Plates 2, 3, and 4 are all replicates.

### Manual Dilutions in 384



mosquito® Dilutions in 1536

## Fluor de Lys Biochemical Assay: Dose Response Curve and EC 50 Comparison



## CONCLUSIONS

We have demonstrated that the mosquito® is capable of producing comparable results when performing serial dilutions in 1536-well microplates. TTP LabTech's mosquito® has the unique additional benefits of a very small footprint and rapid on-the-fly tip exchanging capabilities. This capability has allowed Kalypsys to effectively perform most confirmation, specificity, cytotoxicity, and P450 assays at multiple compound concentrations in 1536-well format, which has further increased the speed and reduced the costs of our drug discovery operation. Performing follow-up assays in 1536 compared to 384-well plates has allowed Kalypsys to fit 2.5 million more compounds on our existing robotic carousels and reduces our compound usage from 10 µL for each 384-well compound hit-pick down to 3 µL's for a 1536-well hit pick. mosquito® has proven to be a practical and cost-effective solution for low volume compound transfer and serial dilution in 1536-well format.