

overview

Examples of mosquito[®] HTS (TTP Labtech) performing miniaturized serial dilution and assay-ready plate preparation in phosphatase activity, and TR-FRET assays; and its comparison to direct dilution performed by Echo 555 (Labcyte Inc.).

introduction

Serial dilutions, plate replications and reformatting into higher density plates are often routine processes in drug discovery laboratories. Miniaturizing these assays have important cost benefits by reducing precious compounds and reagents required, and also allowing for a range of high density plates for higher throughput and efficiency, but these can present challenges for liquid handling systems. As volumes are miniaturized, even small inaccuracies are proportionally magnified. Therefore, it is paramount to choose a reliable and accurate liquid handler.

The mosquito HTS (Figure 1) is a nanolitre to microlitre (25 nL – 1.2 µL) liquid handler enabling automated miniaturization of all standard HTS assays. It offers extremely fast and accurate serial dilution, assay-ready plate preparation, plate replication and reformatting, all in one setting. The disposable tips are based on true-positive displacement, ensuring zero cross contamination and high accuracy and precision for a wide range of liquid viscosities, at nanolitre volumes.

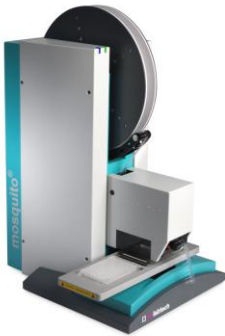


Fig 1: mosquito HTS liquid handler

conclusions

- The use of automated nanolitre and true positive displacement liquid handling technology has proven essential for the assays presented here. The results are rapid, highly reliable and accurate resulting in robust data.
- Transferring only 50 or 100 nL of serially diluted compounds into assay ready plates keeps the diluent (DMSO) concentration at very low levels (0.5%) which is essential for ensuring high integrity of DMSO-sensitive assays.
- Miniaturization of the serial dilution points reduces the amount of compound required.
- All IC₅₀ curves show very tight correlation between the replicate data points, and a great fit of data.
- When comparing the mosquito HTS and Echo 555, the IC₅₀ data clearly demonstrates that the assay performance is equivalent between either dispensing techniques and dilution methods.
- mosquito HTS was proven essential in “wet-dispensing” into 384-well plates, with 50 µL of media already dispensed. Since mosquito HTS does not require the destination plate to be inverted there is no risk of cell media dripping out of the wells, irrespective of well volume or plate type, allowing for live cell screening.

1. comparison of Echo 555 and mosquito HTS

The workflow at the Conrad Prebys Center for Chemical Genomics at Sanford Burnham Medical Research Institute requires the efficient and accurate analysis of a large number of compounds in concentration response follow up studies and during the post ultrahigh throughput screening (uHTS) phase. Therefore these assays are routinely performed in 1536-well format, and hence require the addition of low nanolitre volumes of compounds to the wells.

Insulin resistance is one of the common symptoms in obesity. Low molecular weight protein tyrosine phosphatase (LMPTP) plays a negative role in insulin signaling. Evidence shows that inhibiting LMPTP can reduce obesity associated with insulin resistance. ML400 is an effective inhibitor of LMPTP.

Here, the performance of direct dilution by acoustic dispenser, Labcyte’s Echo 555, and serial dilution using TTP Labtech’s mosquito HTS were compared using phosphatase activity, where a 10-point IC₅₀ curve of ML400 was set up.

methods

A 10-point 1:2 dilution curve was prepared in 1 µL total volume using mosquito HTS and serial dilution wizard (Figure 2). The same curve was set up using direct dilution and Echo 555. 50 nL of the dilution points and controls were dispensed into Greiner 1536-well plates, once into a dry plate and once into 50 µL of media (wet dispense), in replicates. 3-o-methylfluorescein phosphate was used as the fluorogenic substrate to determine the phosphatase in this assay.

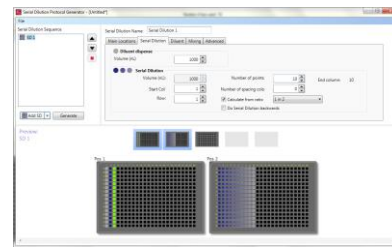


Fig 2: mosquito HTS serial dilution wizard

The assay was repeated twice in 1536-well dry plate with Echo 555 as an established assay protocol. A dry and a wet dispense was tested in 1536-well plates on mosquito HTS for comparison.

results

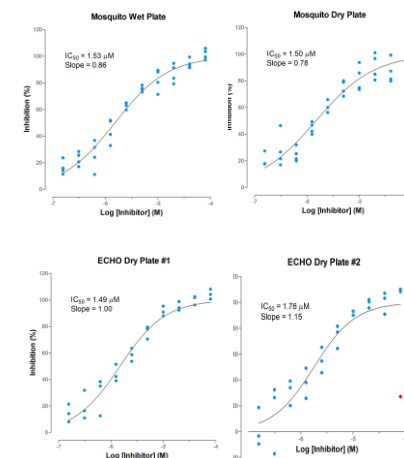


Fig 3: The data shows a tight correlation between serial dilution using mosquito HTS and direct dilution using Echo 555.

2. kinase profiling assay

At ActivX BioSciences Inc. very low volume (1.2 µL) serial dilution points are routinely prepared for kinase profiling assays using the TR-FRET technique. AMC validation data, and IC₅₀ inhibition data from 15 different compounds are presented here.

methods

To validate mosquito HTS, 16 different 1.2 µL 1:3 8-point serial dilution curves were prepared in 384-well plates, and 100 nL of dilution points were stamped into dry 384-well assay-ready plates, in triplicate. The fluorescence intensity was measured for all data points.

For the 15 different compounds, 1.2 µL 1:3 8-point dilution points were prepared using mosquito HTS in 384-well plates followed by stamping 100 nL of the dilution points and controls into dry 384-well assay-ready plates, in triplicate. Then samples were prepared for TR-FRET assay.

results

AMC validation data

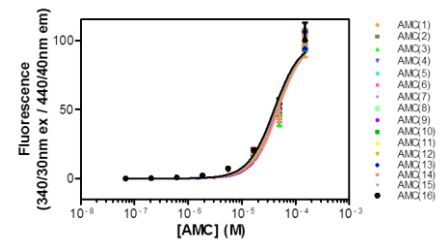


Fig 4: IC₅₀ curves of AMC validation using same compound repeated 16 times.

R² values:

AMC 1	AMC 2	AMC 3	AMC 4
0.9689	0.9723	0.9667	0.9788
AMC 5	AMC 6	AMC 7	AMC 8
0.9764	0.9741	0.9791	0.9766
AMC 9	AMC 10	AMC 11	AMC 12
0.9778	0.9775	0.9757	0.9776
AMC 13	AMC 14	AMC 15	AMC 16
0.9741	0.968	0.9761	0.972

Table 1: The validation data illustrates how precise mosquito HTS is and the reproducibility of an 8-point serial dilution across 16 different channels.

15 different compounds

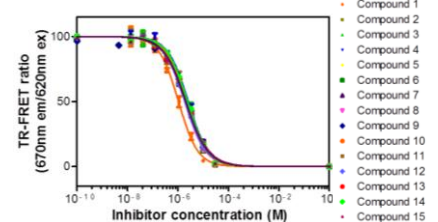


Fig 5: IC₅₀ curves of 15 different compounds.

R² values:

C1	C2	C3	C4	C5
0.9942	0.9920	0.9943	0.9954	0.9931
C6	C7	C8	C9	C10
0.9949	0.9934	0.9936	0.9886	0.9891
C11	C12	C13	C14	C15
0.9913	0.9920	0.9947	0.9917	0.9919

Table 2: The R² values of IC₅₀ data show accurate 8-point serial dilution curves prepared in 1.2 µL for all 15 different compounds.

