

Compound Transfer to 3456-well Assay Plates Using the Echo™550

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Abstract

Many of the HTS assays at Merck are performed in the 3456-well format to enable rapid screening of the sample collection and to reduce the cost of an HTS campaign. Our laboratory has successfully miniaturized many HTS assays to the 3456 format using Aurora Discovery's Flying Reagent Dispenser™ (FRD) and the tcPR™ detector. However, we did not have the capability to transfer 5nL of compound solutions to the 3456 assay plate. The Echo 550 was chosen to perform this task. We will show reproducibility, accuracy and precision data of the 5nL acoustic droplet ejection into the 3456-well plates using fluorescent dyes. Results from a panel of compounds tested in a 3456-well enzymatic assay will also be presented and compared to the original 384-well format.

Introduction

Fig 1A: Labcyte Echo 550™: "Touchless" Nanoliter Pipetting

- Transfer volumes from 2.5 nL to 250nL
- "Touchless" transfer based on acoustic drop ejection
- *In situ* measurement of fluid height and DMSO hydration in microplate wells
- Patented technology



Fig 1B: Echo 550™: Theory

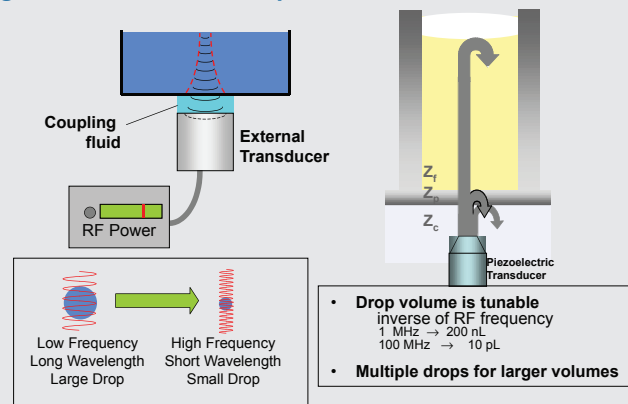
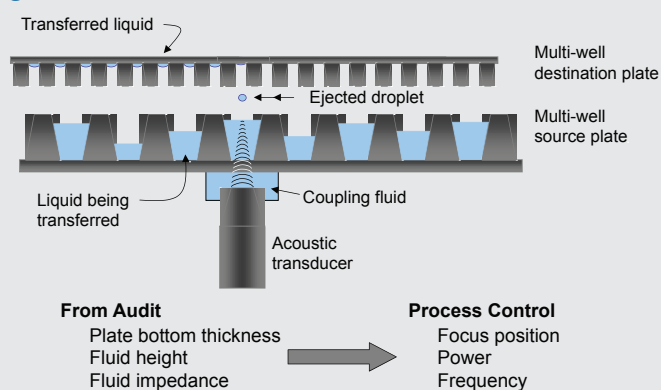


Fig 1C: Echo 550™: Function



Objectives

- Determine accuracy of 5nL dispense into 3456-well plates.
- Wet vs dry dispense of 5nL from 384-well plate to full 3456-well ChemLib plate.
- Determine precision of 5nL dispense from dye concentrations prepared in 70 to 100% DMSO.
- Determine accuracy and precision of 5nL dispense to 3456-well plate using inhibitors in an enzymatic assay.

Materials and Methods

For all experiments

- Source plate: Labcyte ADE certified 384-well PP plate Cat# P-05525-CV1.
- Destination plate: ChemLib 3456-well black COC plate from Aurora Discovery Cat# 17831
- All dispenses were done using the FRD™ and plates were read using the tcPR™ from Aurora Discovery.

Protocols for:

Accuracy Testing and Dry Dispenses

- Added 5nL of Coumarin solution with the Echo550™ + 2µL of 25mM MES buffer pH5.5

Precision Testing and Wet Dispenses

- Added 1µL of 25mM MES buffer pH 5.5
- Added 5nL of Coumarin solution with the Echo550™
- Added 1µL of 25mM MES buffer pH5.5

Protease Enzymatic Assay

- Added 5nL of compound with the Echo550™
- Added 1.6µL of protease in Assay buffer (50mM MES pH5.5, 2.5mM EDTA, 2.5mM DTT, 10%DMSO).
- Incubate 10min
- Added 0.4µL of peptide-AMC (2µM final) in Assay buffer.
- Read in tcPR

Results

Fig 2: Accuracy of 5nL Dispense

- Coumarin standard curve : 2µL of 0 to 8µM Coumarin solution was added into a 3456-well plate with the FRD™.
- Accuracy testing was done by dispensing 5nL of 400µM Coumarin in 100% DMSO for a final concentration of 1µM.

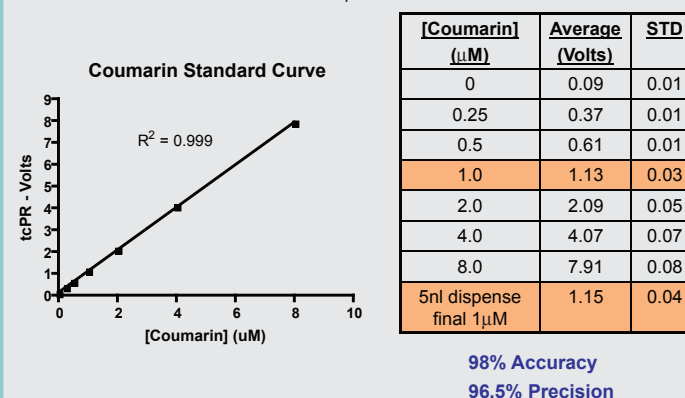


Fig 3: Coumarin Dilution Series - Wet vs Dry Dispense

- Stock solutions of coumarin dye were serially diluted in 70% to 100 % DMSO/water.
- 5nL of the dilutions were transferred from 384-well plate to 3456 plate.
- Centrifuged plate before reading in tcPR

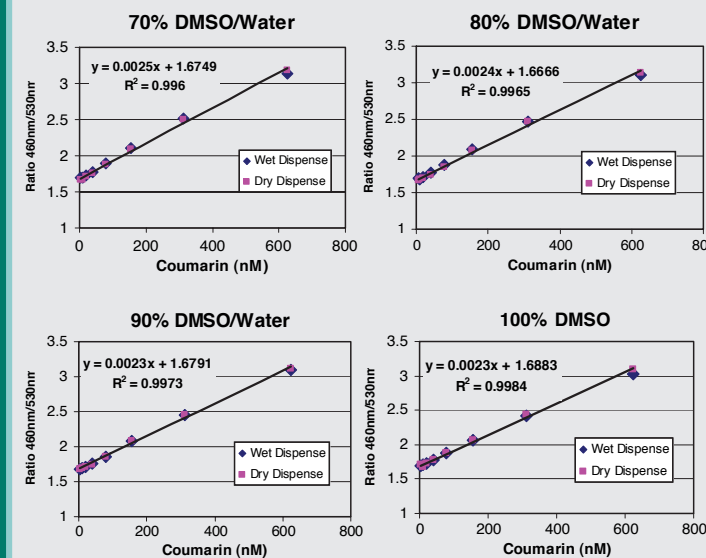


Fig 4: Precision of Dispense - 9x384 to 1x3456

- Precision testing was done by dispensing 5nL of 100µM Coumarin at various %DMSO/water using the wet dispense protocol.
- Read in tcPR @ 460nm & 530nm (normalized background to 530nm)

250nM coumarin per 3456 well	70% (n=864)	80% (n=864)	90% (n=864)	100% (n=864)
Average ratio (Volt @ 460nm / Volt @ 530nm)	2.73	2.69	2.66	2.62
% CV	4.53%	3.42%	4.06%	4.94%

Note: Successfully shot into all 3456 wells.

Protease Assay

- Performed a fluorometric assay (coumarin-labeled peptide) to evaluate the accuracy and efficiency of the 5nL dispense in 3456-well plates using a panel of 29 protease inhibitors.
- 1x384-well plate of serially diluted compounds were transferred 9x into the 3456 plate.
- All other reagents were added with the FRD™
- Plates were read in the tcPR™ for 10 min (total of 4 reads/well).
- IC₅₀ values were calculated based on the rate of fluorescence over the 10 min read interval for each well.

Fig 5A: Titration of 8 Known Inhibitors in the 3456 Protease Assay Format

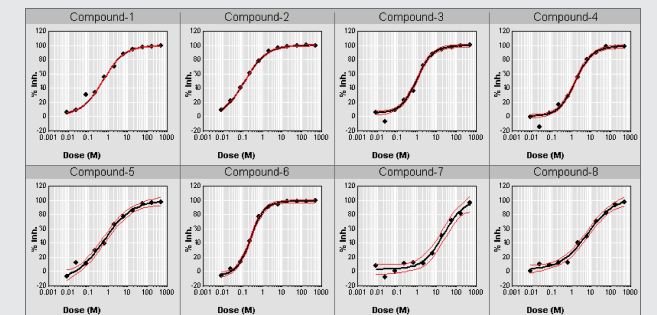


Fig 5B: 9x Replicate Titrations of Same Compound in 3456

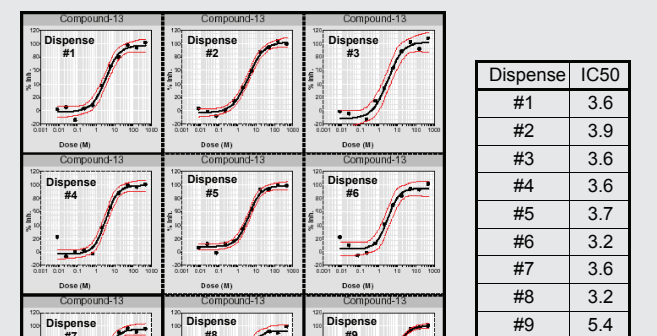
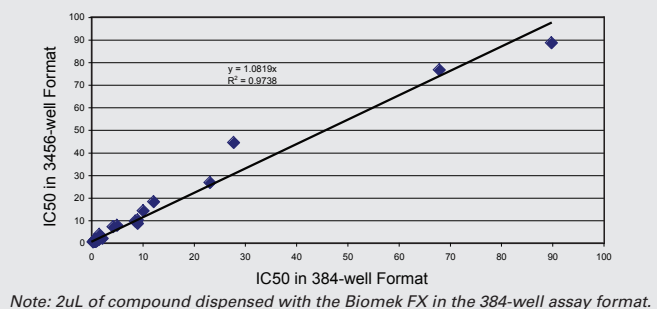


Fig 6: Inhibitor IC₅₀ Comparison Between 384 and 3456 Well Formats



Note: 2µL of compound dispensed with the Biomek FX in the 384-well assay format.

Conclusion

- Echo 550 consistently filled all wells of 3456 plates with 5nL compound/dye (100% successful ejections so far)
- Precise and accurate ejection of 5nL at all DMSO concentrations.
- No difference between wet or dry dispenses.
- Obtained similar IC₅₀ results for protease inhibitors as in the 384-well format.
- No issue with "sticky" hydrophobic compounds (logP values up to 10). LogP values were determined using the ACD algorithm.