

Delivering improved kinetic assay results.

Application Note

**AQUEOUS DISPENSING WITH
THE TECAN D300E DIGITAL DISPENSER.**



INTRODUCTION

The Tecan D300e Digital Dispenser offers an unprecedented dispensing range for both small molecules in DMSO and biomolecules in aqueous solution – including proteins, DNA, lipids, and nanoparticles – simplifying dispensing of test compounds and reagents directly into assay plates. Its large dynamic range, enabled by non-contact dispensing of liquid volumes as low as 11 pl, allows rapid set-up of dose-response experiments without the need for serial dilution. This is particularly useful for co-titration of aqueous solutions of enzymes, substrates and inhibitors in enzyme profiling experiments.

MATERIALS

D300e Digital Dispenser and T8+ Dispensehead cassettes

- Dispense range: 11 pl to 10 μ l
- Plate formats: 1,536, 384, 96, 48, 24, and 12 wells
- Plate height: 6-47 mm
- Compound waste: 0.5-4 μ l, depending on experiment
- DMSO fluid space: 70-100 % DMSO
- Aqueous fluid space:
- Biomolecules up to 300 kDa and up to 3 mg/ml in surfactant-containing solutions
- Small molecules solutions up to 10 mM containing surfactant
- Nanoparticles in suspension at a concentration <0.5 % containing surfactant
- Surfactant concentration requirements:
 - Brij™ 35; 0.10 %
 - Triton™ X100; 0.10 %
 - Tween® 20; 0.30 %
- Optional additive:
 - Glycerol; 0-20 %

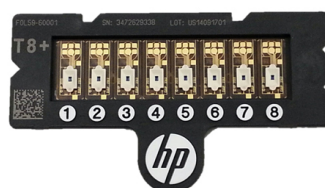


Figure 1: D300e Digital Dispenser and T8+ Dispensehead cassette

Aqueous dispensing capabilities

The D300e with T8+ cassettes has excellent dispensing accuracy and precision (low %CV) across a range of dispense volumes. It can dispense aqueous liquids at volumes as low as 11 pl (13 pl for DMSO) and demonstrates CVs of <8 % for dispense volumes greater than 100 pl. Aqueous dispensing requires the addition of small amounts of surfactant, and solutions can also optionally include up to 20 % glycerol.

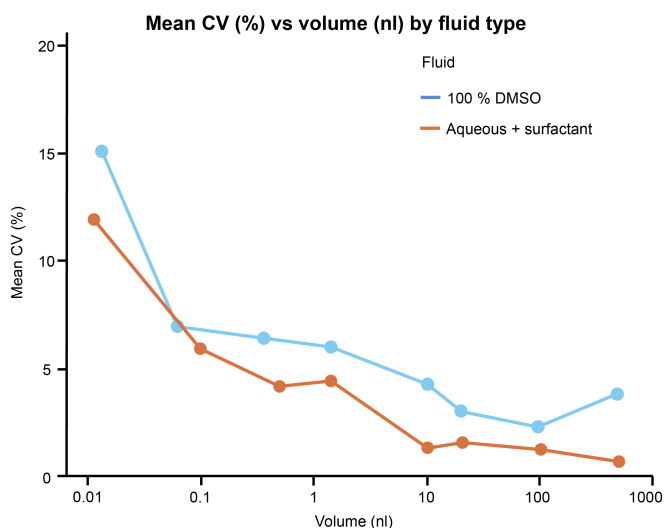


Figure 1: D300e dispensing precision with DMSO and aqueous fluids at various volumes.



Enzyme profiling: wizard set-up

The D300e's intuitive software permits easy set-up of difficult enzyme profiling experiments. Enzymes, substrates, cofactors and inhibitors can be added in combination at different concentrations, easily accomplishing even the most challenging experiments.

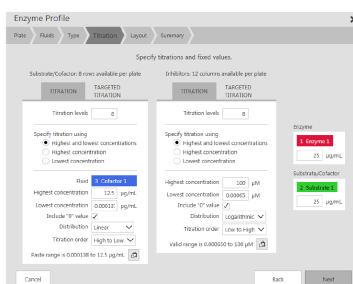
Select fluids



Select desired treatment



Set up titrations, concentrations



Select layout

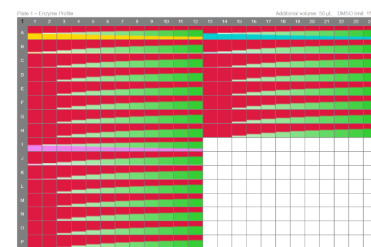
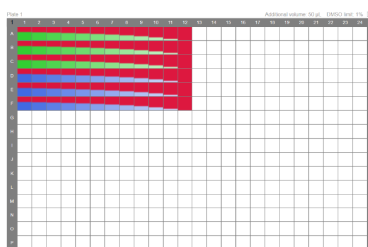
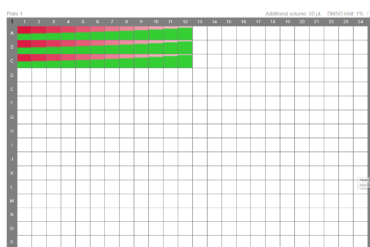
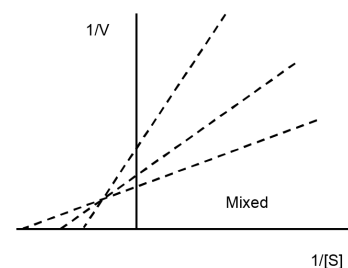
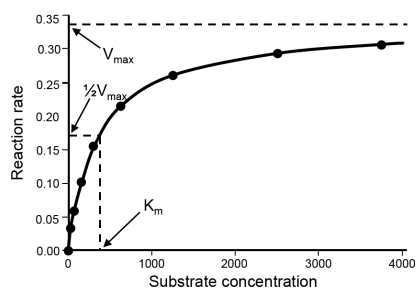
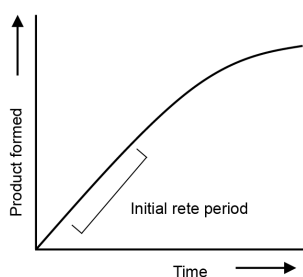
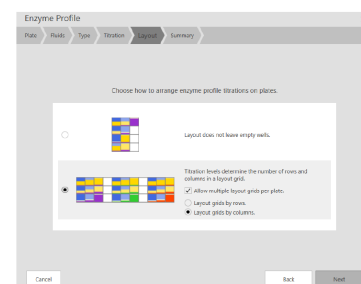


Figure 2: The enzyme profile wizard takes users through the process step by step to create enzyme characterization, K_m determination and inhibition mechanism studies.

RESULTS

Histone deacetylase (HDAC) kinetics and K_m determination

Aqueous stock solution (75 $\mu\text{g/ml}$) of HDAC and substrate were dispensed using the D300e and T8+ cassettes. The 2:1 enzyme dilution series was simple to create and yielded the optimal enzyme concentration and time course for the K_m determination. The subsequent K_m determination, shown in a Michaelis-Menten plot (Figure 3), was consistent with previous experimental data. Set-up of the entire assay was completed in less than 15 minutes, representing an enormous time saving over other methods.

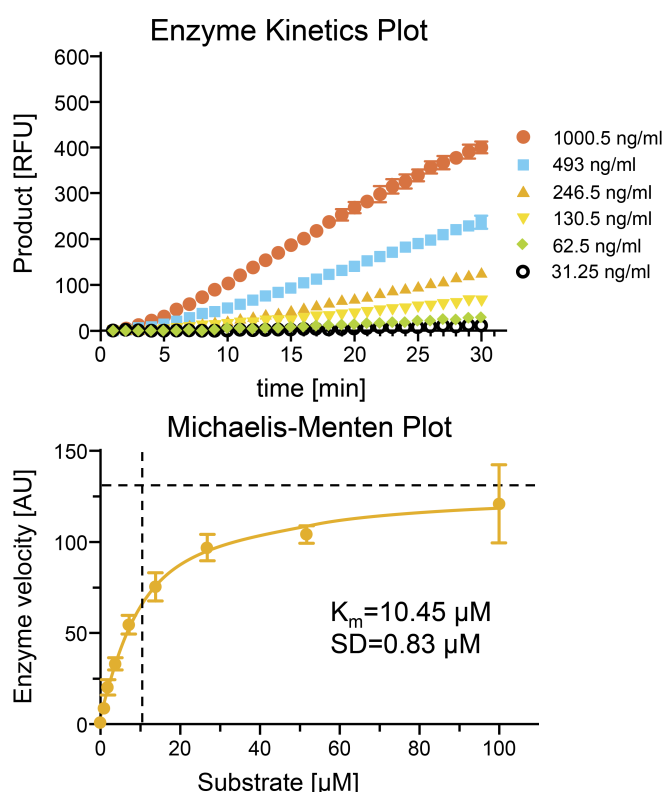


Figure 3: Aqueous enzyme and substrate dispensed with the D300e and T8+ cassettes. Data courtesy of Dr Ralph Mazitschek, Massachusetts General Hospital.

Identification of inhibition mechanism

Substrates in aqueous solution and inhibitors in DMSO were dispensed for enzymatic assays using the D300e and T8+ cassettes. Lineweaver-Burk analyses were performed for two substrates and indicated mixed-mode inhibition of enzyme in the presence of substrate 1, and non-competitive inhibition in the presence of substrate 2. Determination of this type of competitive mechanism is particularly difficult to accomplish with classical techniques, but is readily achieved with the D300e, due to its ease of programming and capability to perform digital, non-contact dispensing to every well.

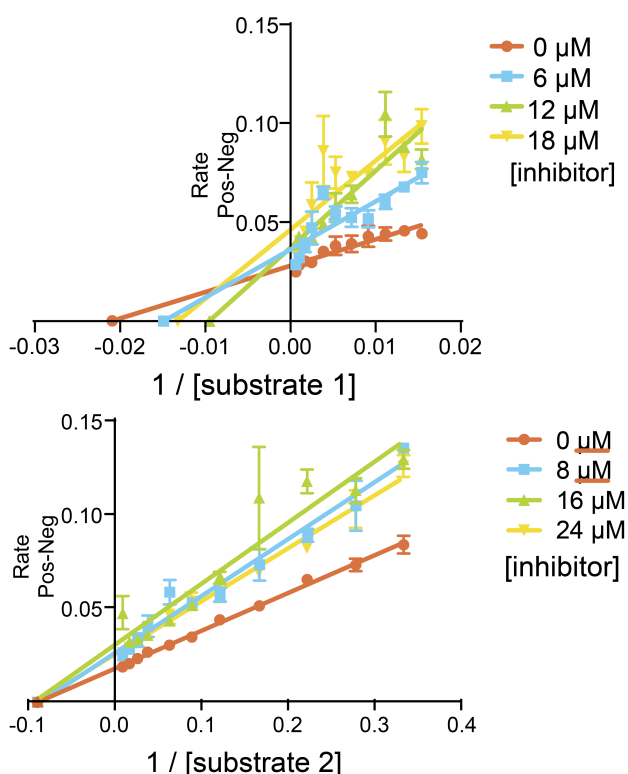


Figure 4: Co-titration of aqueous substrates and inhibitors in DMSO for inhibition mechanism studies by enzymatic assays.

SUMMARY

Using the new T8+ cassettes, the Tecan D300e Digital Dispenser offers outstanding dispensing precision, simplified workflows and best-in-class ease of use, significantly improving assay performance for both DMSO- and aqueous-based experiments. The data presented in this application note underlines the suitability of the T8+ cassettes for protein-based assays.



About the author

Dr. Manuel Bauer joined Tecan Switzerland as a product manager in 2013 and is responsible for the Tecan D300e and the options portfolio. He studied Biology at the University of Würzburg and Free University of Berlin. During his PhD at the ETH Zürich he focused on systems biology and has also applied various proteomics techniques during his post doc at the Biozentrum Basel.



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