Evaluation of the Formulatrix Tempest at EMD Serono: A flexible low volume liquid handler with two axis gradient dispensing capability

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ABSTRACT

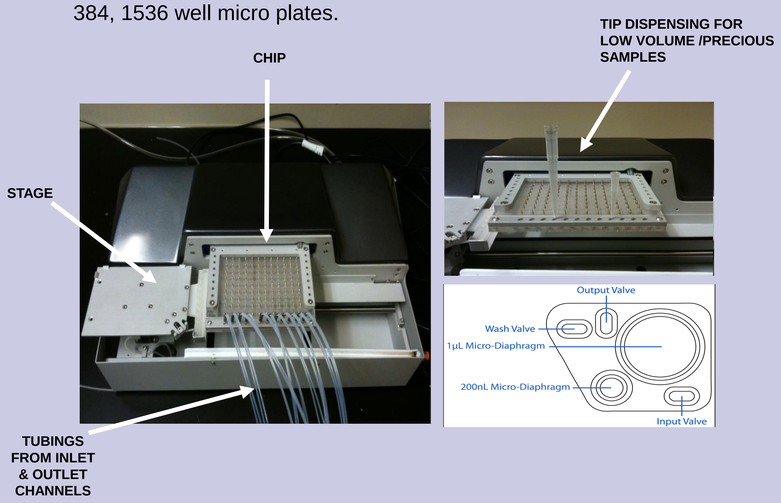
Liquid handling instrumentation which provides precise and flexible dispensing over a range of volumes and different components is core to a fully functional assay development, hit discovery and characterization lab. At EMD Serono, we use liquid dispensing (50 ul to 200 nl) in a limited number of tasks, such as regular assay component dispensing (around 15-70 ul), as well as low volume dispensing (200 nl). Other aspects of assay development & biochemical characterization of inhibitors are performed in a manual and labor intensive manner.

Introducing the Formulatrix Tempest in our workflow has allowed us to improve the current technique not only in terms of efficiency but also accuracy and precision. Unique to the Tempest is its new microfluidic technology that relies on independent channel control over all the 96 nozzles, thereby allowing one to dispense any volume from any input into any well. We evaluated this diversity for a wide range of assays from those requiring factorial dispensing, like assay design and modality of inhibition studies, to applications like assay miniaturization that use fixed volume dispensing.

Through this poster we show the robustness and flexibility that the Tempest provides compared to other liquid handlers

TEMPEST

The Tempest is a flexible and compact liquid dispenser that uses state of the art microfludics to dispense any volume from any input into any well. It uses positive displacement to dispense liquids with minimal waste. It has a dispensing range of 200nl to no upper limit and a very low non-recoverable dead volume (~40ul). Typical dead volumes are ~400ul and can be reduced to ~100ul using tip dispensing. It is accurate, precise, fast and is compatible with 96, 384, 1536 well micro plates.



TEMPEST ACCURACY

We analyzed the accuracy of the Tempest in two formats

a) A fixed volume miniaturized assay comprising of 2ul enzyme & 3ul substrate.

b) A variable volume assay that requires different volumes of 8 different enzymes.

5ul Assay		
Avg	Std dev	CV
0.390	0.003	0.818
0.388	0.003	0.896
0.390	0.003	0.739
0.389	0.003	0.805
0.392	0.004	0.993
0.390	0.004	1.018
0.390	0.004	0.950
0.387	0.004	0.974
0.390	0.003	0.754
0.390	0.003	0.714
0.389	0.003	0.801
0.389	0.003	0.789
0.387	0.003	0.761
0.386	0.003	0.755
0.388	0.004	1.011
0.386	0.003	0.866

Variable Volume Assay (CV's)	
Kinase I	1.318
Kinase P	1.122
Kinase A1	1.133
Kinase F	1.296
Kinase I	1.508
Kinase P	1.550
Kinase A1	2.519
Kinase F	2.374
Kinase FL	1.885
Kinase R	3.109
Kinase S	2.109
Kinase V	1.905
Kinase FL	1.820
Kinase R	1.584
Kinase S	1.721
Kinase V	2.085

With both formats, we found the CV's for the whole plate to be within the acceptable range of 5%.

WORKFLOW COMPARISION

Our current workflow for establishing assay parameters involves 3 separate steps.

a) Titration to determine the enzyme concentration for a 30% conversion. (Fig 1)

b) Kinetic run to determine the ATP Km of the enzyme. (Fig 2)

c) Final titration to confirm the established parameters. (Fig 3)

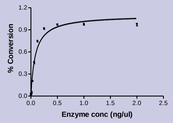


Figure 1 : Kinase A enzyme titration at 100uM ATP

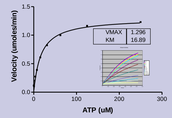


Figure 2: Kinetics for Kinase A

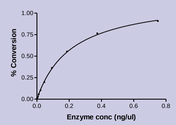
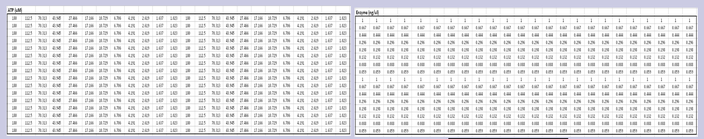


Figure 3: Kinase A enzyme titration at 15uM ATP

These resource intensive steps can be eliminated with the Tempest, which by virtue of its independent channel control allows a factorial dispense of ATP and enzyme. The resulting matrix design enables us to attain the assay parameters in a single step (Fig 4,5,6).



35% conversion @ 17uM ATP and 0.13ng/ul enzyme.

ATP (uM)

ENZYME (ng/ul)

	180	112.5	70.3	43.9	27.5	17.2	10.7	6.7	4.2	2.6	1.6	1.0
1	0.968	0.971	0.964	0.958	0.909	0.872	0.775	0.683	0.575	0.362	0.249	0.149
0.667	0.919	0.913	0.900	0.872	0.805	0.723	0.646	0.530	0.448	0.264	0.200	0.107
0.444	0.954	0.955	0.943	0.929	0.866	0.803	0.693	0.585	0.468	0.287	0.187	0.107
0.296	0.879	0.875	0.846	0.816	0.718	0.625	0.540	0.429	0.325	0.206	0.140	0.082
0.198	0.697	0.678	0.637	0.592	0.484	0.446	0.327	0.252	0.199	0.131	0.091	0.066
0.132	0.621	0.590	0.564	0.511	0.497	0.349	0.277	0.206	0.160	0.107	0.081	0.059
0.088	0.276	0.255	0.223	0.209	0.155	0.155	0.112	0.080	0.061	0.053	0.044	0.042
0.059	0.279	0.255	0.221	0.203	0.156	0.124	0.105	0.076	0.060	0.050	0.043	0.042

Figure 4: Percent Conversions for Kinase A (Averages from all 4 quadrants)

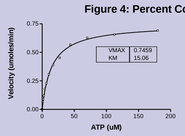


Figure 5 : Kinetics for Kinase A (TEMPEST)

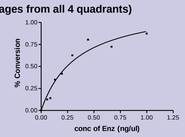
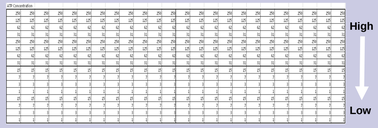


Figure 6 : Kinase A enzyme titration at 15uM ATP (TEMPEST)

MODALITY OF INHIBITION

We evaluated the Tempest's efficiency in modality of inhibition experiments. By dispensing a gradient of ATP down the plate we studied the response of two in-house competitive inhibitors.



Factorial Dispensing using TEMPEST

DATA

Figures 7 & 8 show IC50 curves for inhibitors (A & B) at distinct ATP concentrations. IC50 values at other concentrations is given in table 1.

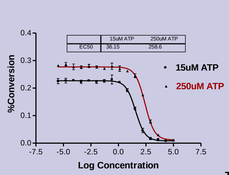


Figure 7 : Inhibitor A at 15 and 250 uM ATP

Inhib A IC50 (nM)	ATP (uM)	Inhib B IC50 (nM)
258	250	1953
151	125	1300
91	62.5	882
65	31.25	530
36	15	402
27	7.5	337
21	3.75	258
10	1.75	119

Table 1: IC50 values of both inhibitors at various ATP concentrations

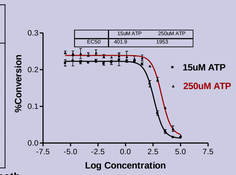


Figure 8 : Inhibitor B at 15 and 250 uM ATP

It can be seen from Fig 9 and 10 that for both Inhibitor A and Inhibitor B, the potency of inhibition decreases with increase in ATP concentration, a characteristic of Competitive Inhibition.

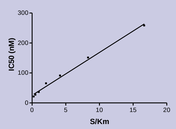


Figure 11 : Competitive inhibition of Kinase A by Inhibitor A

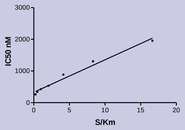


Figure 12 : Competitive inhibition of Kinase A by Inhibitor B

CELL DISPENSING

We further evaluated whether operation of the diaphragm during dispensing had an effect on cells. As shown in Fig 13 & 14, we saw no morphological changes in cells dispensed with the Tempest when compared to manual dispense.

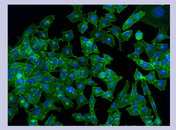


Figure 13 : A375 cells dispensed manually

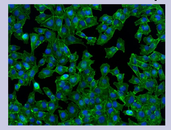


Figure 14 : A375 cells dispensed using Tempest

SUMMARY

Low volume pipetting technology is key to our workflow and is of utmost importance. Through this poster we have highlighted the improved efficiency the Tempest provides to our workflow. Other key features include an easy USB connectivity, a user friendly software and automation capability. ELISA, PCR, HTS, DNA/RNA research are some of the other applications that the Tempest can support.