

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

Vikram Shankar*, Adam Shutes, Bill Griffin, Brian Healey

Lead Discovery Technologies, EMD Serono Research Institute, Rockland, MA, 02370. *vikram.shankar@emdserono.com

ABSTRACT

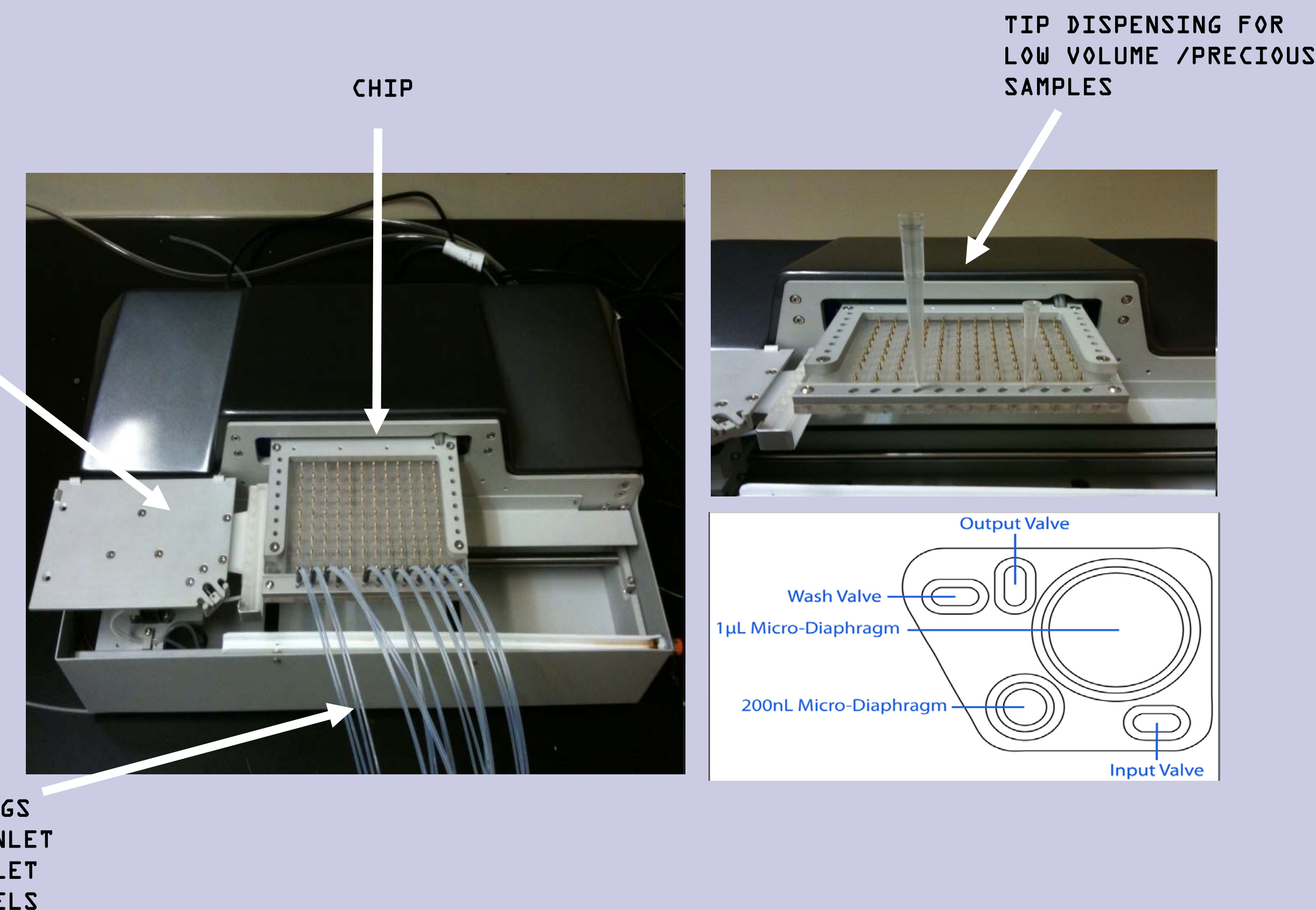
Liquid handling instrumentation which provides precise and flexible dispensing over a range of volumes and different components is core to a fully functional assay development, hit discovery and characterization lab. At EMD Serono, we use liquid dispensing (50 ul to 200 nl) in a limited number of tasks, 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 microfluidics 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.

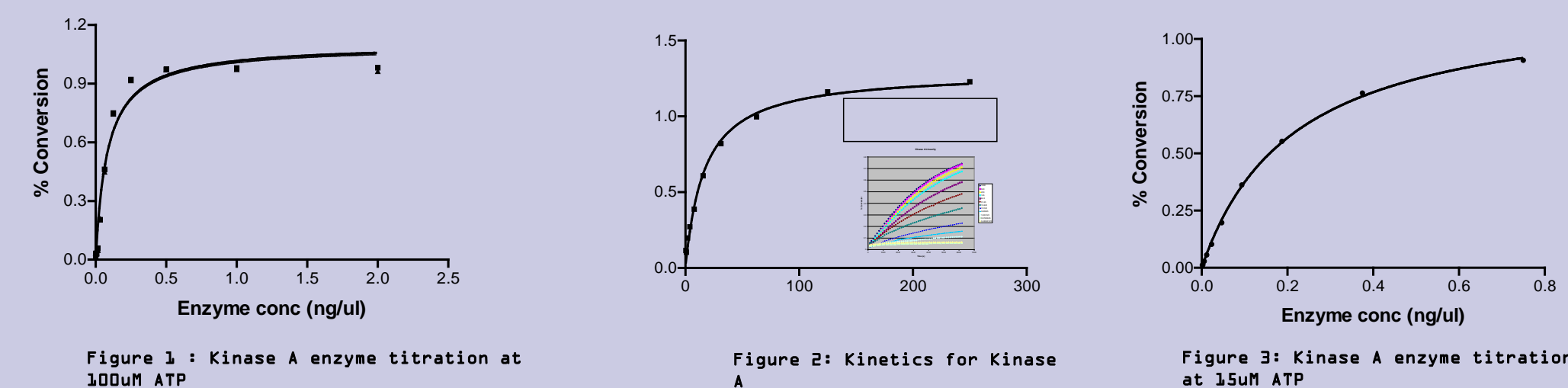
5ul Assay			Variable Volume Assay (CV's)	
Avg	Std dev	CV	Enzyme	CV's
0.390	0.003	0.818	Kinase I	1.318
0.388	0.003	0.896	Kinase P	1.122
0.390	0.003	0.739	Kinase A1	1.133
0.389	0.003	0.805	Kinase F	1.296
0.392	0.004	0.993	Kinase I	1.508
0.390	0.004	1.018	Kinase P	1.550
0.390	0.004	0.950	Kinase A1	2.519
0.387	0.004	0.974	Kinase F	2.374
0.390	0.003	0.754	Kinase FL	1.885
0.390	0.003	0.714	Kinase R	3.109
0.389	0.003	0.801	Kinase S	2.109
0.389	0.003	0.789	Kinase V	1.905
0.387	0.003	0.761	Kinase FL	1.820
0.386	0.003	0.755	Kinase R	1.584
0.388	0.004	1.011	Kinase S	1.721
0.386	0.003	0.866	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 COMPARISON

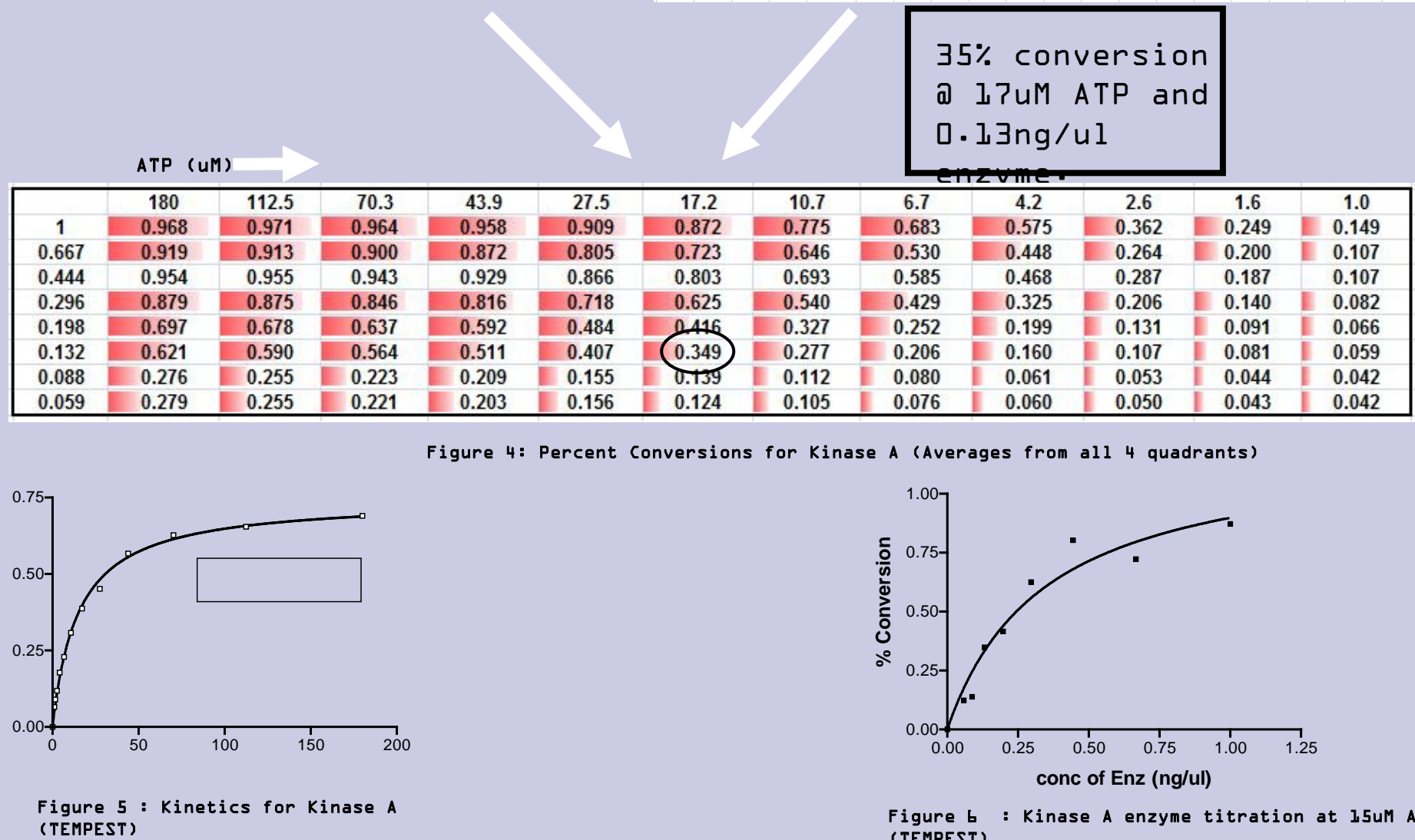
Our current workflow for establishing assay parameters involves 3 separate steps.

- 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)



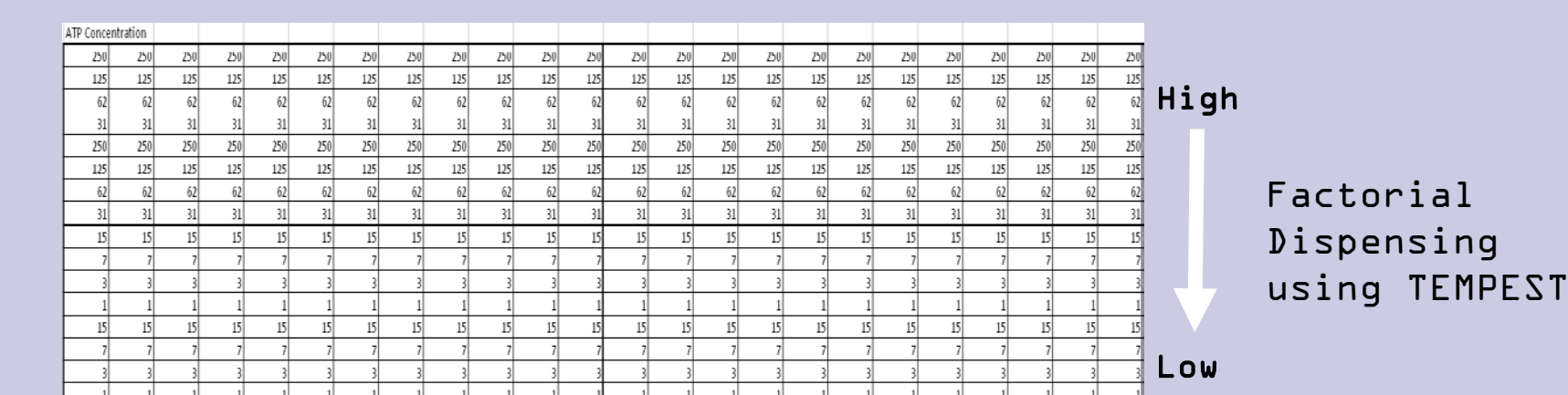
These resource intensive steps can be eliminated with the Tempest, which by virtue of its independent channel control allows a factorial dispense of ATP and enzyme. The resulting matrix design enables us to attain the assay parameters in a single step (Fig 4,5,6).

ATP (uM)	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	0.408	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	0.197	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	0.857	0.859	0.837	0.802	0.684	0.592	0.496	0.377	0.252	0.169	0.131	0.091
0.132	0.621	0.590	0.564	0.511	0.407	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	0.112	0.080	0.061	0.053	0.044	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



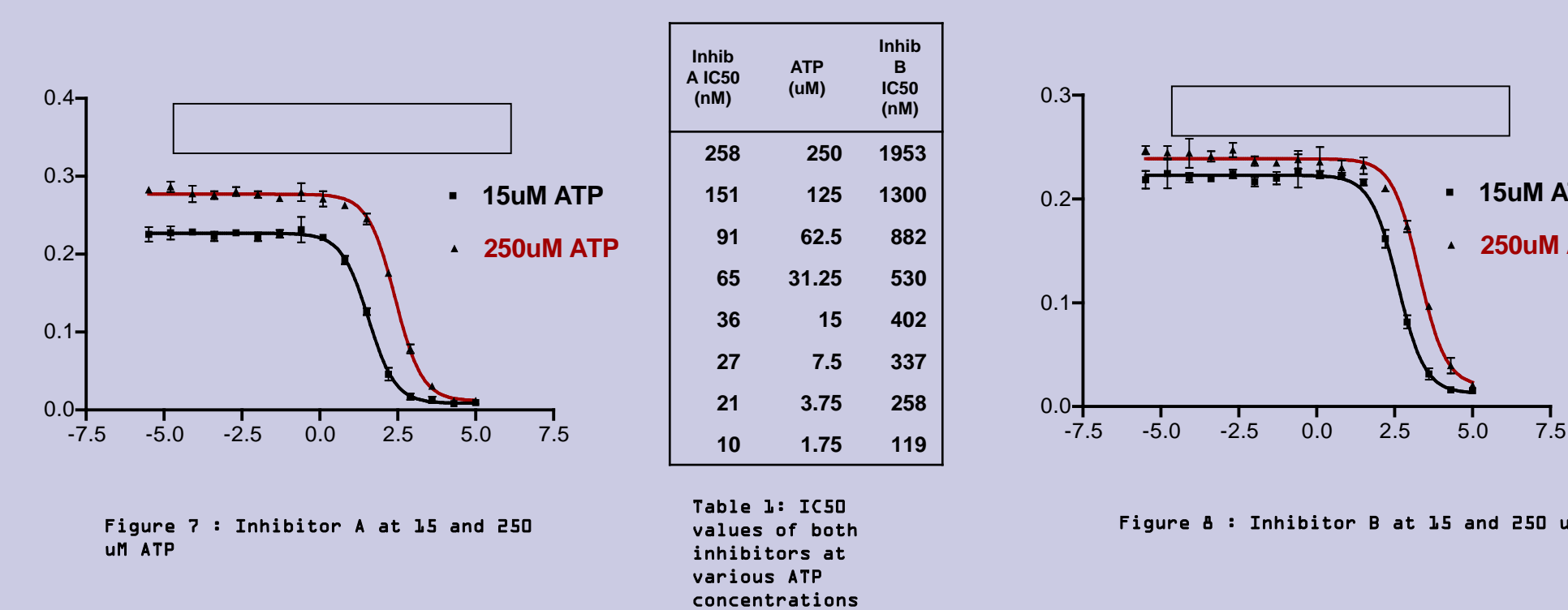
MODALITY OF INHIBITION

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.

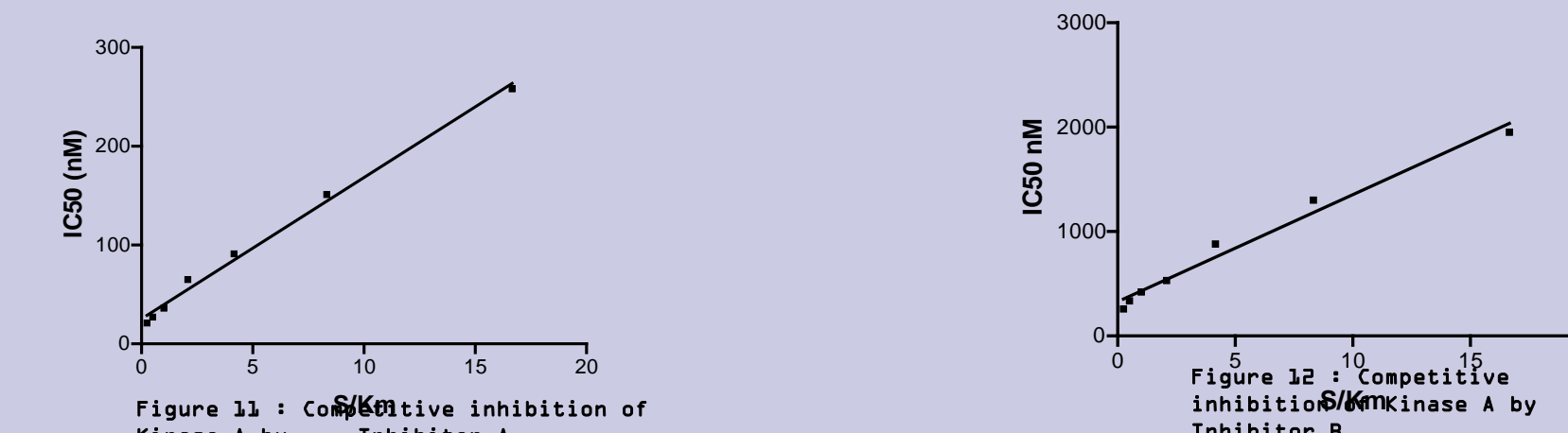


DATA

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

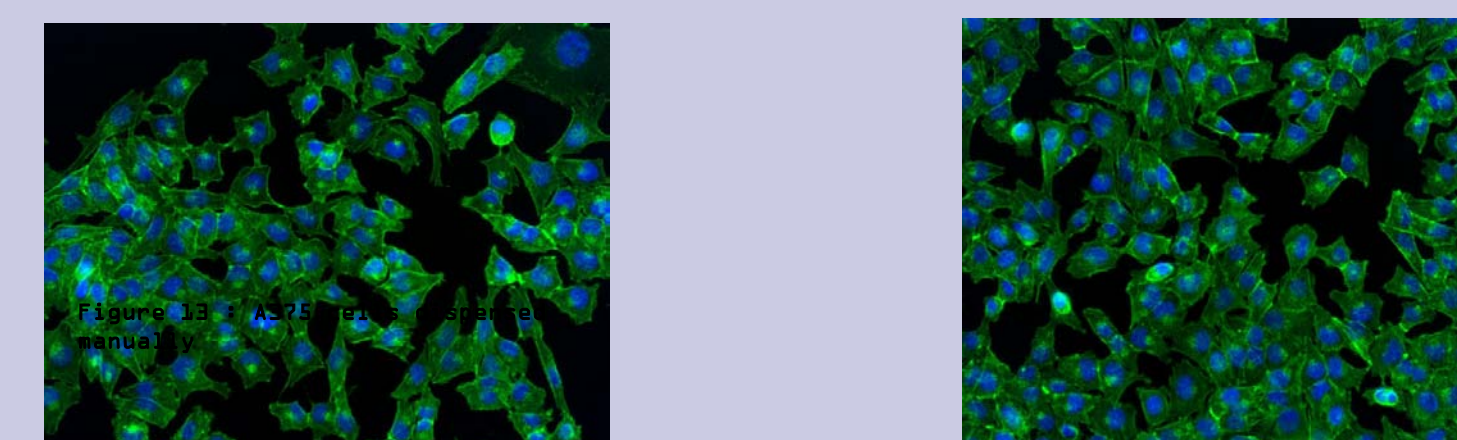


It can be seen from Fig 9 and 10 that for both Inhibitor A and Inhibitor B, the potency of inhibition decreases with increase in ATP concentration, a characteristic of Competitive Inhibition.



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.



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.