Three Different Liquid Handling Systems to Achieve Flexibility and Scalability in Compound Screening against 400+ Protein Kinases

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ABSTRACT

AssayQuant Technologies has developed a chelation enhanced fluorescence (CHeF) assay that enables the screening of proprietary sensor peptides to monitor the activity of an individual kinases in a continuous assay format. AssayQuant leverages this technology in its KinSight™ profiling service – a kinome screening platform that can evaluate a single compound against a panel of greater than 400 kinase targets. To meet the increasing demands of these unique selectivity profiling services, the automation team at AssayQuant developed a robust, automated plating process that accounts for several factors: 400+ kinases currently in the profile, two different ATP conditions at which kinase assays are routinely performed (1mM and Km), and special assay conditions that specific kinases require for adequate enzymatic activity. The automation team considered throughput and reliability in an automation platform as well as instrument redundancy to guarantee that high-quality data collection, analysis, and reporting are completed with accuracy and efficiency.

Three automation systems were designed and implemented to accomplish these requirements. Inventory management and labware standardization was established to allow for uniform plating of all assay reagents (kinases, sensor peptides, compounds, and assay buffer components) on any liquid handler. The plating protocol was designed with cherry picking capabilities to enable additional flexibility and thus allow for assays to be run with ATP at 1 mM or at each individual kinase's Km. Overall, the three systems implemented differ in deck capacity, throughput, and required user intervention, however, when combined they ensure redundancy and allow for increased overall throughput.

THE *PHOSPHOSENS*® TECHNOLOGY – CONTINUOUS & ENDPOINT/RED FORMATS

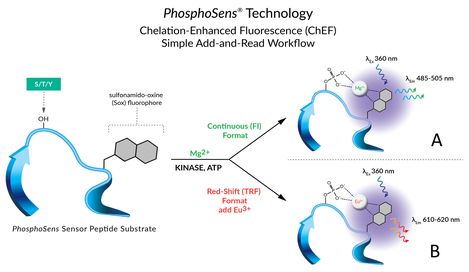


Figure 1

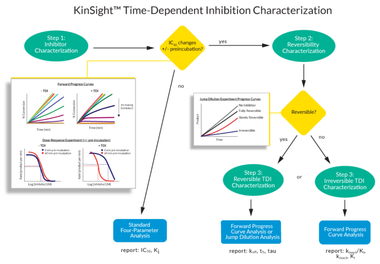


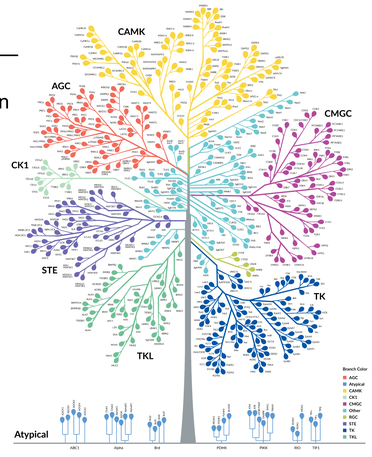
Figure 2

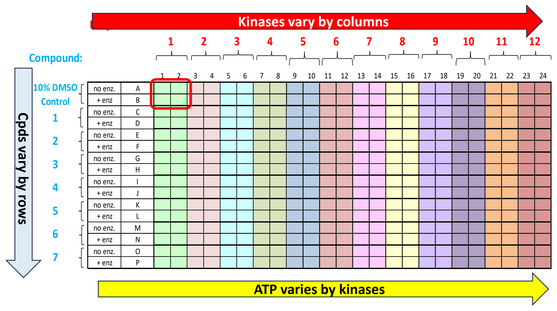
Figure 3

PhosphoSens Sensor Peptide substrates are synthesized using solid-phase methods with the Sox fluorophore coupled through the sulfhydryl group of a cysteine residue proximal to a protein kinase phosphorylation site, such as a tyrosine, serine or threonine. Upon addition of a kinase, the peptide is phosphorylated. In the presence of magnesium ion, a chelation complex is formed with the phosphate group, resulting in fluorescence enhancement of the Sox fluorophore that can be **monitored continuously** as fluorescence intensity **(A)**. Kinase inhibitors prevent phosphorylation and thus fluorescence. At any point, Europium ion can be added, to displace the magnesium ion, resulting in a long wavelength, time-resolved fluorescence (TRF) **endpoint/Red format (B)** that is useful for high-throughput or structure activity relationship (SAR) applications (Fig 1). The technology has been incorporated into Custom Testing services (Fig 2) as well as the Catalog (Fig 3).

KINSIGHT™ PROFILING

AssayQuant's KinSight quantitative kinome profiling services are powered by our novel and proprietary ***PhosphoSens*® technology** — a continuous assay format. This assay directly quantifies the enzymatic activity of the target by reporting substrate phosphorylation during the entire reaction. A continuous format yields an actual reaction rate determined from dozens of data points, generating a progress curve in every well.



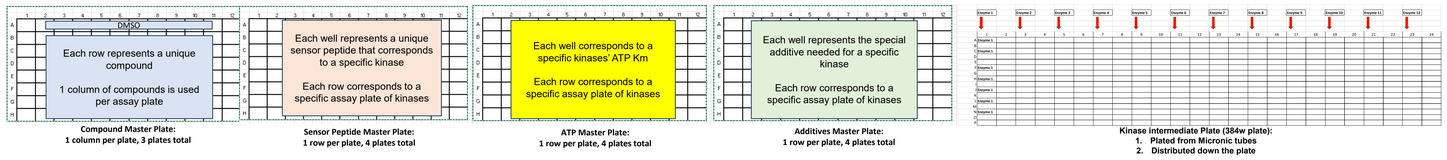


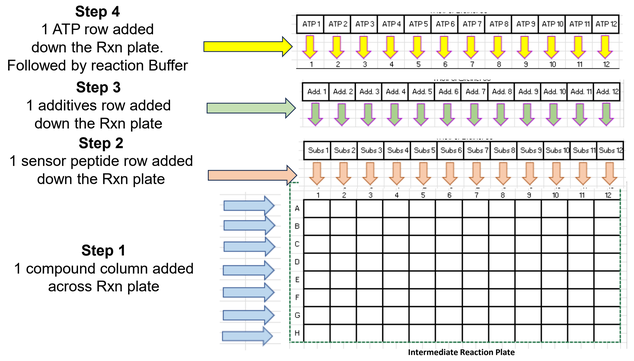
KinSight Profiling

- Sensor Peptide-Kinase pairing for 400+ wt kinases
- ATP at 1mM or Km
- 34 assay plates per KinSight Profile Panel
- 12 Kinases and 7 compounds per assay plate
- 324 kinases require standard assay conditions
- Remaining kinases require special assay reagents
- ATP needs to be added separately due to Km conditions
- Kinase handling requires plates to be created just-in-time (JIT)

Automation needs to be able to add reaction buffer, ATP, compounds, special reagents & kinases quickly, consistently

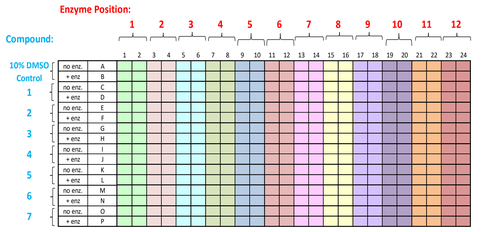
Standardizing the Workflow





- **Kinase addition must be last due to the sensitivity of kinase activity**

5. Create Kinase intermediate plate
6. Stamp intermediate reaction plate
7. Stamp Kinase Buffer
8. Stamp Kinase plate
9. Start Kinetic Assay



Kinsight Profile Panel Assay Plate

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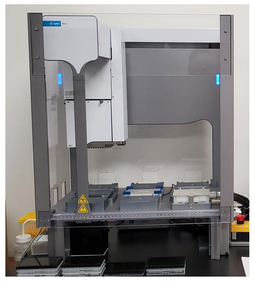
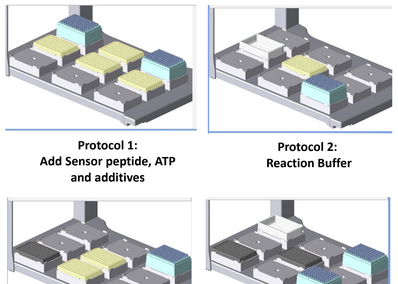
Three Ways to Achieve Compound Screening

"Manual" Processing: Agilent Bravo



Agilent Bravo with 96LT



Protocol 1: Add Sensor peptide, ATP and additives

Protocol 2: Reaction Buffer

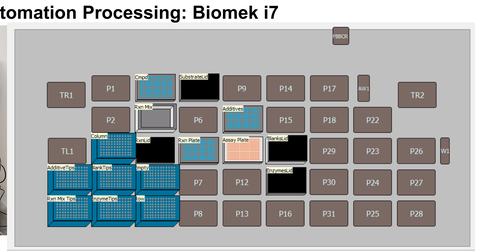
Protocol 3: Add Cpds

Protocol 4: Add Enzyme

- Agilent Bravo with 96LT Head, 9 deck positions available
- Bravo limited deck space requires process to be segmented into four protocols.
- Requires an operator to continually change the protocol and load labware ("Manual")

Partial Automation Processing: Biomek i7

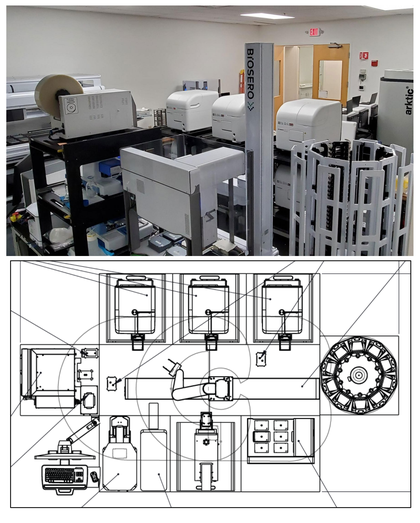
Biomek i7



I7 Deck layout

- I7 with 96 head, 384 head and Span8
- Deck capacity allows for all tips and assay reagent to be placed on the deck at one time.
- One method distributes all components (sensor peptide, ATP, compounds, reaction buffer and kinase)

Full Automation: BioSero Kinase Screening Workcell



- Precise Automation PF400 robot, PAA CS-10 Carousel, Cytomat Incubator, 6 Synergy Neo 2 readers, Biosero GBG Software
- Agilent Bravo with 96LT Head, 8 deck positions available, 1 trash chute
- Deck limitations require four protocols to add all components to an assay plate.
- Precise robot arm manipulates reagents on and off the Bravo deck
- GBG monitors reader activity and regulates the plating schedules
- GBG adjusts plating schedule if a reader is in error state

"Manual" Bravo

- "Manual" Bravo protocols take 4 minutes/protocol to complete, for a cumulative time of 16 minutes per assay plate.
- "Manual" Bravo requires the shortest time to complete a full Kinsight panel however is the most labor intensive as an operator must constantly be available.
- Compounds need to be run at either ATP 1mM or ATP Km.
- Method available to split volumes in half to conserve kinases and consumables, requires 16 Synergy readers

Biomek i7

- Using the i7, each plate take 18 minutes to complete.
- An operator is required for the setup of each plate when using the i7
- Compounds can be run at ATP 1mM, Km or a mix of ATP concentrations.
- Utilize 9 Synergy readers

Biosero Kinase Screening Workcell

- The Precise robot arm removes the limitation of the Bravo deck and constant operator intervention.
- Day mode operation – Protocol pauses until operator intervenes
- Night mode operation – Errant readers are removed from scheduling and automation continues.
- Compounds need to be run at either ATP 1mM or ATP Km.
- Workcell requires to be setup twice during a full panel run.

SUMMARY

- KinSight Profiling screens compounds against up to 400 different wild type kinases per profiling panel. 34 assay plates per KinSight Profile Panel
- The utilization of Master plates allows for KinSight to be performed at either 1mM ATP or Km
- Each automated platform utilizes the same Master plates to generate the assay plates.
- Three automation systems enables flexibility and scalability with regards to turnaround time and capacity.
- Flexible and agnostic to screening contents. New kinases can be incorporated readily with minimal method modifications needed.
- Phosphatases or kinase mutant screening can be incorporated with in the KinSight Panel or as a new service using the same methods.
- Custom Kinase panels employ the same strategy irrespective of the number of kinases in the custom panel