Plasma-based Cleaning of Pipette Tips Used in an Automated Kinase Assay

Introduction

A kinase is an enzyme that actively removes a phosphate group from high-energy donor molecules such as ATP, then covalently attaches it to amino acids bearing free hydroxyl groups (specifically serine, threonine or tyrosine). Most kinases are selective for either serine-threonine or tyrosine laden sites for phosphorylation, while several kinases react with all three amino acids. The act of phosphorylation increases the overall energy of a target molecule (substrate) and provides the 'catalyst' for a substrate to participate in a subsequent reaction. In other instances, phosphorylation causes molecules to undergo conformational changes, thus enabling or limiting the overall activities of both substrate and kinase, resulting in 'self-regulation'.

Kinases constitute about 2 percent of all eukaryotic genes, and actively modify upwards of 30 percent of all proteins in an organism. The human genome contains roughly 500 protein kinases that act on small molecules such as lipids, carbohydrates, amino acids and nucleotides. Kinases regulate the majority of cellular pathways and play a vital role in signal transduction via cell signaling or downstream biochemical reaction.

Plasma Cleaning of Pipette Tips

Kinase activity (Figure 1) in eukaryotes is generally viewed as one of the most important and central physiologic processes, the deregulation of which is arguably a major root cause for disease since kinases regulate many aspects that control cell growth, cell movement and death. Consequently, small molecules which inhibit specific kinases are constantly being developed to treat several diseases, with some currently in clinical use.

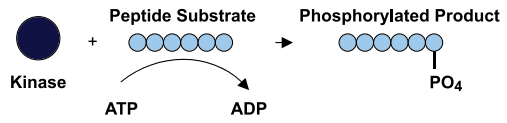


Figure 1. *The kinase-driven phosphorylation reaction. Kinase in the presence of ATP results in the binding of a phosphate (PO_4) group in the peptide substrate.*

The TipCharger™ by IonField Systems™ uses self-contained, low-temperature, atmospheric plasma to clean pipette tips, metal cannula and pin tools associated with automated liquid handlers. Implementation of the TipCharger system provides cleaning equivalent to that of a fresh set of tips, reducing both the direct and indirect costs associated with replacing pipette tips. Using the TipCharger system, plasma was validated as a tool for effectively eliminating carryover using a well characterized and fully optimized automated kinase assay.

Further testing revealed that implementing TipCharger cleaning protocols in place of traditional tip replacement practices provided equivalent IC_{50}s for a variety of kinase inhibitors, thereby reducing the number of tip boxes necessary for screening.

TipCharger Integration

The TipCharger is provided in 8, 96 and 384-well plate densities and is easily integrated into most liquid handling platforms using standard SBS footprints. In this study a Sciclone ALH 500 (Caliper Life Sciences, Hopkinton MA) was used in conjunction with a TipCharger TC-96 cleaning station taught as a reagent reservoir. Contaminants on the exterior of pipette tips exposed to TipCharger-generated plasma are immediately ionized; contaminants inside pipette tips are removed through a series of aspirate and dispense steps while tips are in the cleaning station.

General Screening Conditions

Compounds (10 mM, 100% DMSO) in wells A2 through A11 of a 96-well mother plate were serially diluted by volume 1:3 (Falcon #353072) with DMSO. Control wells also received DMSO. The contents of the mother plate were further diluted in an intermediate plate (Falcon #352191) using assay buffer for a working concentration of 200 µM (4% DMSO). Compound or DMSO was added to the corresponding wells in the reaction plate with the highest final concentration of 10 µM at 0.2% DMSO and subsequent lower concentrations based on the serial dilution.

Kinase (45 nM) was added to each well of a 96-well reaction plate (Matrix #4936) with the exception of the negative control wells (A1-H1) which received assay buffer. The peptide substrate (5 µM) solution containing 125 µM ATP was added to all wells, shaken for 5 minutes at room temperature (RT) and then incubated for 60 minutes at 37°C.

The reaction was terminated through the addition of a detection buffer containing 20 mM EDTA, LANCE Eu-labeled anti-phosphotyrosine antibody (1 nM) and Streptavidin conjugated APC (300 nM). Assay plates were shaken for 30 minutes at RT and read on an Analyst Microplate Reader (Molecular Devices, Sunnyvale CA) with an excitation wavelength of 330 nm and emission wavelengths of 620 nm and 665 nm for donor and acceptor respectively.

Each assay well was performed in triplicate across three separate reaction plates.A single box of Caliper 100 µL polypropylene automation pipette tips (part number #105647) was used per reagent addition and replaced thereafter (Table 1).

Kinase Reaction	
Component	Volume dispensed/well, Time
Compound	5 µL
Change tips	30 seconds
Peptide solution	10 µL
Change tips	30 seconds
Kinase solution	10 µL
Change tips	30 seconds
Incubation	60 minutes @ 37° C
Stop Solution	35 µL
Optimized Pipette Tip Plasma Exposure - Kinase Assay	
Component	Volume dispensed/well, Time
Compound	5 µL
TipCharger Step	10x Mix, 100 µL/ 100 µL second
Peptide solution	10 µL
TipCharger Step	10x Mix, 100 µL/ 100 µL second
Kinase solution	10 µL
TipCharger Step	10x Mix, 100 µL/ 100 µL second
Incubation	60 minutes @ 37° C
Stop Solution	35 µL

Table 1. *Study set- up.*

Validating TipCharger in Automated Kinase Assays

Peptide containing ATP (125 µM) was added to all wells of six, 96-well reaction plates (Matrix #4936). Kinase (45nM) was added to columns 2-12 of three 96-well plates and an equivalent volume of kinase buffer was added to negative control wells. Tips were dirtied by aspirating and dispensing kinase and then cleaned via a 20 second mix protocol inside the TipCharger cleaning station. The same set of tips was used to add kinase buffer to experimental Plate 4 in an effort to elute any remaining kinase.

This process was repeated for each of the other two experimental plates. The remaining steps were completed as described under the General Screening Conditions. The entire process was repeated for peptide by plating kinase first and then following the remaining steps including those specific to the TipCharger cleaning station.

Results & Conclusion

A 20 second exposure to atmospheric plasma generated in the TipCharger is sufficient for reducing kinase from pipette tips to levels similar to that of the negative control (Figure 2). In addition, the same result was achieved when the peptide substrate was eliminated from tips.

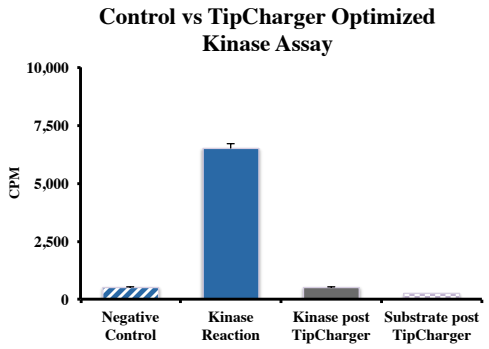


Figure 2. *Pipette tip plasma exposure results for kinase and substrate.*

Exposure to plasma showed no significant kinase assay activity and restored pipette tips to a state similar to that of a new set of tips. In addition to kinase and substrate, a range of small molecule kinase inhibitors were eliminated via plasma exposure subsequent to rinsing with dH_2O (data not shown). This rinse step can lower DMSO percentages prior to tip insertion in the TipCharger cleaning station and enhance the life of the cleaning station. Further experiments demonstrated that re-use of TipCharger plasma-cleaned pipette tips resulted in data indistinguishable from that obtained in protocols demanding continual tip replacement. The sigmoidal curves resulting from both methods are equivalent as shown in Figures 3 and 4.

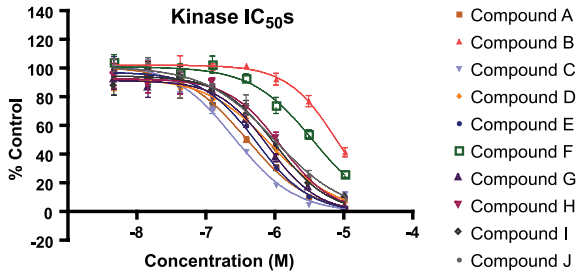


Figure 3*. Normal dose response curves for a variety of kinase inhibitors (250 nM - 10 µM) with continual tip replacement.*

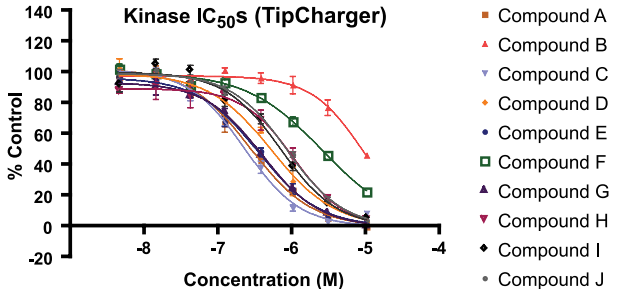


Figure 4. *Dose response curves for a variety of kinase inhibitors (250 nM- 10 µM) using a single box of tips per assay.*

No significant difference was observed in the calculated IC_{50}s between normal screening conditions and TipCharger optimized conditions (Table 2).

	Control IC_{50}	TipCharger IC_{50}
Compound A	383	264
Compound B	7440	9120
Compound C	247	221
Compound D	863	716
Compound E	525	445
Compound F	3390	3480
Compound G	779	785
Compound H	1180	1020
Compound I	978	893
Compound J	948	945

Table 2 *Comparison of IC_{50}s with and without TipCharger use.*

Summary

Screening for small molecule kinase inhibitors remains an important component of the drug discovery environment. The ability to clean pipette tips to states equivalent to that of new pipette tips , without negatively impacting an assay, enables screening environments to increase throughput and reduce the cost of screening campaigns. Collectively, the time saved using plasma pipette tip cleaning increased assay throughput overall and resulted in a tip cost reduction of nearly 95%. Given that drug discovery environments are making every effort to reduce time-to-market for drug candidates, the TipCharger system may play a significant role in achieving this objective.

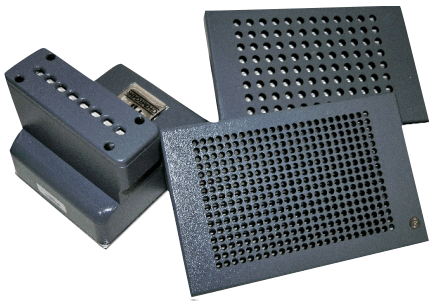
Integrating the TipCharger into Automated Assays Provides the Following Benefits:

Best in Class TipCharger cleans better than any other washing technology - in most applications the TipCharger will clean to background, so there is no difference between plasma cleaning and a new tip.

Cost Benefit TipCharger saves over 90% on the cost of the disposable tips.

Speed Incorporating the TipCharger System can result in a time savings of 10-30 seconds for every microplate processed or rack of tips cleaned.

Convenience Clear away the clutter and save time: Integrating the TipCharger System eliminates the need to store cases of new pipette tips and dispose of racks of hazardous used tips.



TipCharger Plasma Cleaning Stations
Available in 8, 96, 384, and 1536 channel versions

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About the TipCharger™

The TipCharger Cleaning System replaces existing wash stations and easily integrates with most existing and new automation platforms. The system utilizes a low temperature, atmospheric pressure plasma process that cleans metal and plastic pipette tips and pin tools. Treated surfaces are clean, dry and have uniform surface properties.

The TipCharger cleaning process reduces the incidence of micro-bubble formation and other random surface effects that degrade liquid handling precision and accuracy, even with new disposable tips.

IonField Systems' TipCharger improves the reproducibility of process results, shortens automation cycle times, reduces the number of lost runs, and eliminates environmental waste and liquid handling disposables. The overall result is increased confidence in results and a more effective and productive laboratory operation.

About IonField Systems™

IonField Systems is an advanced technology company focused on the development of best-in-class cleaning methods for life science applications.

We provide comprehensive onsite/ phone support and ongoing services to insure a successful installation and exceptional day-to-day operation of our products.