Significant Improvement in the Throughput of Automated cAMP Cell-Based Assay via Plasma-based Cleaning of Pipette Tips

Introduction

Cyclic AMP (cAMP), as a secondary messenger, plays a critical role in a number of intracellular pathways. Often used as a proxy for potentiated hormone responses, steroid metabolism, kinase activation, and G-Protein-Coupled Receptors (GPCRs) binding, quantitation of cAMP is an essential tool for the drug discovery industry. As a result, there are a number of commercial kits available for the detection of cAMP as a biomarker, most available in forms optimized for automated analysis. The efficacy of using the TipCharger™ by IonField Systems™ plasma technology for cleaning pipette tips was demonstrated with a well characterized cell-based assay for the identification of small molecule inhibitors for a specific GPCR via cAMP production.

Plasma Cleaning of Pipette Tips

The TipCharger 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.

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. The TipCharger can be taught as either a device or consumable within the liquid handler software.

In this study, the TipCharger TC-96 cleaning station was taught as a reservoir using an automated liquid handler with 100 µL polypropylene tips. Contaminants on the exterior of pipette tips exposed to TipCharger-generated plasma are immediately ionized; contaminants inside the pipette tips are removed through a series of aspirate and dispense steps while tips are in the cleaning station. Carryover was compared to normal cAMP detection/ production levels using the Amersham Biosciences cAMP SPA Biotrak Direct Screening Assay System (#RPA 559) (Figure 1).

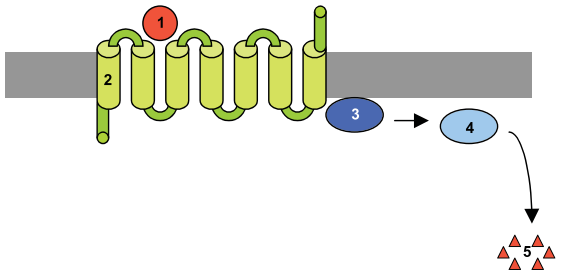


Figure 1. *Assay specific cAMP mechanism of action. (1) A ligand binds to the cell surface receptor (2) driving transducer activity. (3) resulting in primary effector activity (4) and production of the cAMP secondary messenger (5)*

Note: None of the reagents specific to the cAMP kit were tested against the TipCharger cleaning station.

General Screening Conditions

Trypsinized HEK 293 cells were plated in 96-well, Poly-D-Lysine coated tissue culture plates (BD Biosciences #35-6651) at a density of $2.0x10^5$ cell/mL and incubated for 16 hours at 37°C, 5% CO_2 and 95% humidity. A reference inhibitor (10mM, 100% DMSO) in wells A12 through H12 of a mother plate was serially diluted by volume 1:3 in DMSO across the plate (Falcon #353072). DMSO was added to column 1 for controls.

The contents of the mother plate were further diluted in an intermediate plate (Falcon #352191) containing 1X PBS, 100µM IBMX to form a highest working concentration of 10µM (0.1% DMSO). Next, tissue culture media (DMEM, 10% hi-FBS, 1% MEM, and appropriate selection antibiotics) was removed and replaced with assay buffer containing the reference inhibitor. Cells were incubated (37°C, 5% CO_2 and 95% humidity) for a predetermined period of time. Agonist was added to a final concentration of 3 nM and cells were again incubated (Table 1).

Normal Cell-Based Assay		
Component	**Volume per Well**	**Incubation time**
Assay Buffer w/ Compound/DMSO	100 ul	15 minutes
Agonist	5 ul	15 minutes

Assay w/TipCharger Integration		
Component	**Volume per Well**	**Incubation time**
Assay Buffer Post Agonist Exposure and TipCharger	100 ul	15 minutes
Agonist	5 ul	15 minutes

Table 1: *Volume and Incubation Times for the TipCharger evaluation.*

Finally, cells were lysed using the lysis buffer provided in the Amersham cAMP detection kit and the remaining steps specific to the kit were followed. The assay plates were incubated at room temperature for 16 hours and read on a Wallac 1450 TriLux microbeta counter (PerkinElmer, Wellesley MA). A standard curve was generated in parallel to determine cAMP levels.

TipCharger use in Automated cAMP Cell-Based Assays

Agonist was added in duplicate to the appropriate wells across two 96-well cell plates. Next, a fresh set of tips was exposed to agonist through aspirate and dispense steps of equal volume to that added to the first set of plates. Tips were then inserted into the TipCharger cleaning station for a predetermined period (6 seconds).

The same set of tips was used to aspirate assay buffer in place of agonist and added to the second set of cell plates (the process was repeated individually per cell plate). Both sets of plates were placed in a tissue culture incubator for 15 minutes. Cell plates were then processed using the protocol and reagents provided in the Amersham cAMP assay kit (Figure 2).

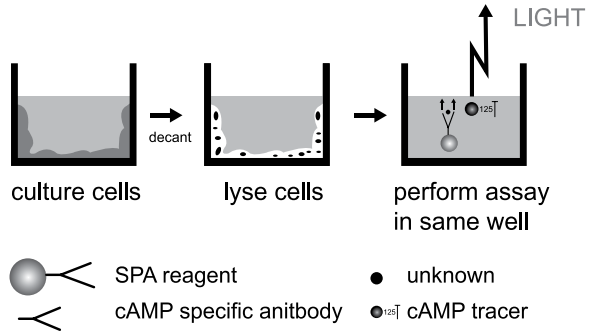


Figure 2. *Amersham BioSciences cAMP SPA BioTrak Direct Assay System.*

Results and Conclusions

An control inhibition curve was generated when agonist was added to cells after incubation with a increasing dose of a reference inhibitor. However, exposing the agonist to the TipCharger plasma cleaning technology prior to addition to the cells eliminates agonist activity as expected (Figure 3).

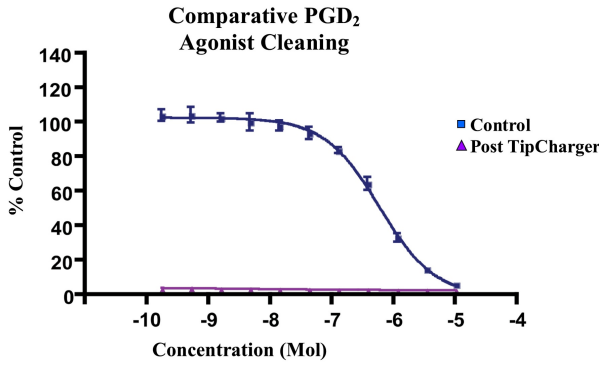


Figure 3. *Dose response inhibition curves without and without TipCharger plasma exposure.*

A comparative analysis of agonist-driven cAMP response shows that the TipCharger System is capable of reducing the cellular response equivalent to an absence of agonist (Figure 4).

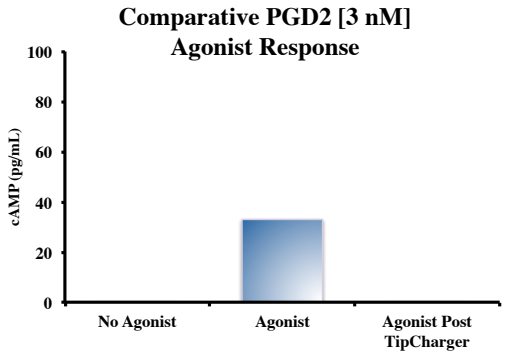


Figure 4. *Comparative analysis of agonist-driven cAMP response with and without using the TipCharger plasma cleaning technology.*

The results show that the TipCharger is an effective and reliable alternative to constantly replacing pipette tips on an automated liquid handler.

Summary

The cAMP assay evaluated in this study was fully-automated in order to decrease screening timelines and provide walk-away operation for the user.. The need to constantly deliver tips to the liquid handling system resulted in logistic bottlenecks and frequent delivery and loading errors. These issues, limited the utility of the fully automated assay.

The integration of the TipCharger system resulted in a n efficient reconfiguration of the cAMP cell-based assay. The ability to achieve equivalent, if not further improved, data quality without the need to change sets of tips resulted in a near-doubling of assay throughput.

Implementing the cold plasma cleaning technology utilized by the TipCharger system within automated laboratories offers opportunities to deliver better assays as well as increase the efficiency and productivity of liquid handling processes.

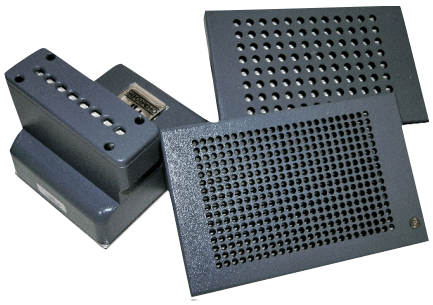
Integrating the TipCharger into Automated Assays Provides the Following Benefits:

Confidence 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 can save up to 98% on the cost of the disposable tips and extends the life of fixed 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 and 384 channel versions

Headquarters
IonField Systems
One Executive Drive
Suite 8
Moorestown, NJ 08057 USA

Call: +1.856.437.0330

Fax: +1.856.823.1426

Email: info@ionfieldsystems.com

www.ionfieldsystems.com

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 state-of-the-art pipette tip cleaning for life science laboratory research applications. IonField Systems provides on-site technical support services to assist laboratories in rapidly integrating the system into day-to-day operations.