Maximizing the Precision and Accuracy of a Multi-Channel Pipettor to Optimize Inhibition Data from a Kinase Assay

Hudson ROBOTICS, INC.

Abstract

The ADP-Glo luminescence assay from Promega is best run at the five microliter scale and consists of three components that are added in one and two microlfiter additions. Such an assay cannot be run in a reproducible manner using a standard multi-channel handheld pipettor with a supported range of 2-20 microliters.

However, attaching the pipettor's head to Hudson Robotics' proprietary motion control increases its precision by an order of magnitude.

The actual pipetting method employed greatly affects the precision of low volume transfers, and a number of pipetting methods were examined using an iterative gravimetric technique developed to measure these effects. The most precise of these pipetting methods were applied to the ADP-Glo assay.

Hudson SOLO Automated Pipettor

Hudson developed a robot to control the movement of a standard multi-channel manual pipettor. The robot contains stepper motors to move the pipettor in the x, y and z-axes, plus an additional motor to control the movement of the plunger. This motor operates at a resolution of roughly 10,000 steps to a full stroke. This translates to 500 steps per microliter.

The unit can be connected to a computer system through a USB-to-serial convertor, and is controlled by SoloSoft software.

Hudson was interested in developing an automated protocol for running Promega's ADP-Glo kinase assay (See next section). This assay includes a range of liquid transfers from 1 to10uL.

It was expected that the high resolution of the motor controlling the plunger would maximize the precision of all of these transfers.

The ADP-Glo Kinase Assay

ADP-Glo is a universal kinase inhibition assay that measures the amount of ADP generated. It follows a simple 3-step protocol that includes:

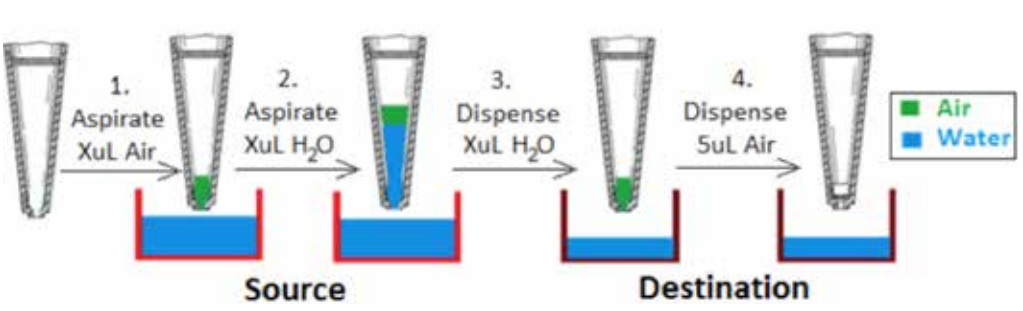
1. Run a kinase reaction (enzyme/substrate/ATP/buffer) with (or without) inhibitor present.
2. Add ADP-Glo reagent: Quenches the reaction and removes all unreacted ATP.
3. Add Kinase detection reagent: Converts the remaining ADP to a measurable luminescence signal

The luminescence directly indicates the effectiveness of the inhibitor being tested.

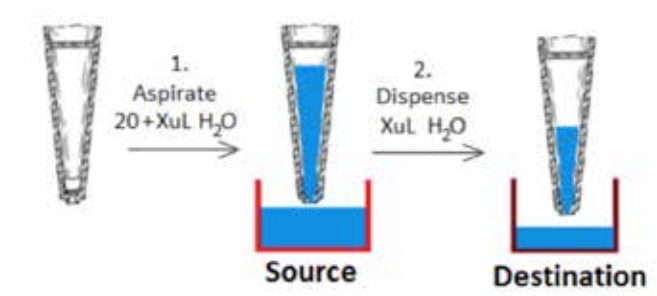
Pipetting Methods

1. **Direct Aspirate/Dispense** – This is the most basic method in which the pipette aspirates and then dispenses a given amount. This accuracy of this method is problematic at low volumes, mostly due to liquid remaining in the tip.

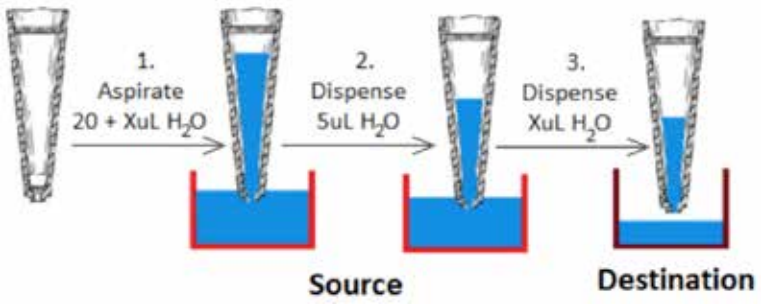
2. **Pre-Aspirate/Blow-Off** – This is the same as Method 1 but is preceded by aspirating a volume of air, followed by a blow-off of the same volume. The blow-off improves the accuracy of this method, but the increased ratio of air/liquid in the system results in reduced precision. This method most closely mimics that achieved with a standard hand-held pipettor.

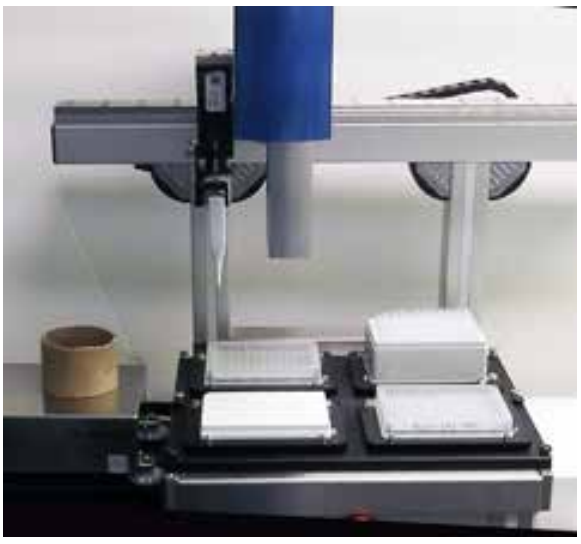


3. **Over-Aspirate** – This is similar to method 1 but uses a larger aspirate amount. This approach combines the benefit of Method 1 (decreased air vs Method 2) with the benefit of Method 2 (all desired liquid leaves the tip). As a result this method gives excellent accuracy and precision.



4. **Over-Aspirate/Pre-Return** – This is similar to Method 3 but with a small return of liquid to the source before the actual dispense step. It was determined that the switch from upward plunger movement (aspiration) to downward movement (dispense), includes a small "backlash" effect that is only noticeable below 5uL, but can be eliminated by the initial dispense to source





Gravimetric Testing

A A simple automated gravimetric method was developed to determine the precision and accuracy of the SOLO using several different parameters at various volumes.

The track on which the pipette head moves extends several inches past the side of the SOLO, allowing movement of liquids beyond its deck. Readers, heating/cooling/vacuum nests, washers and dispensers have been placed in this position. In this study, a Sartorious microbalance was placed in this position, as shown in Figure 2.

A plug-in was written to allow communication between SoftLinx (Hudson's lab automation software) and the microbalance. This plug-in allows forthe tare to be balanced between experiments and the weights retrieved after the liquid is dispensed.

A SoftLinx protocol was created that iteratively zeroes the balance, runs a SoloSoft procedure, and stores the resulting weight in a table. After 20 replicates, the numbers were averaged and CV and standard deviations were calculated.

Results

This gravimetric procedure was carried out for each variation of interest. The volume dispensed, the pipetting method applied, and the type of disposable tip were varied. The results are summarized below:

Figure 3. Dispense Precision as a Function of Pipetting Method #1 vs #2.

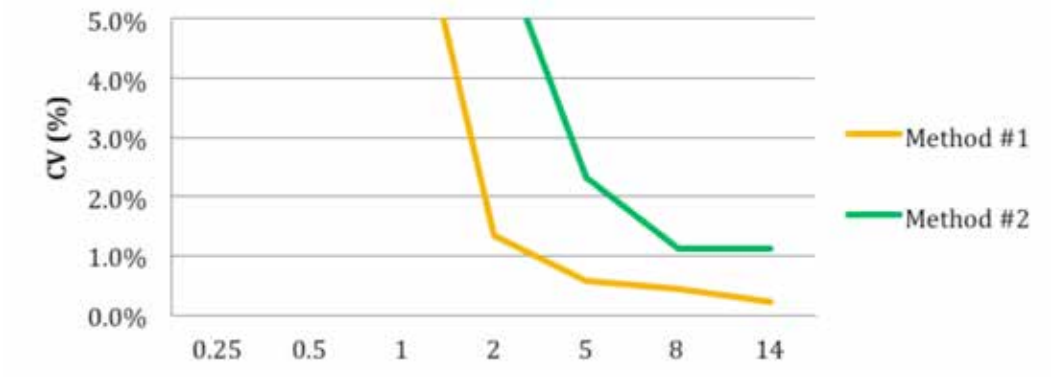


Figure 3 shows the effect of pre-aspirate and blow-off on pipetting precision. Whereas a pre-aspirate and blow-off generally improves accuracy at low volumes, it clearly has a negative impact on precision. CVs become unacceptable using this method below 5uL, whereas direct aspirate/ dispense gives better CVs down to 2uL, both methods are unacceptable for dispensing lower volumes.

Figure 4. Dispense Precision as a Function of Pipetting Method #3 vs #4.

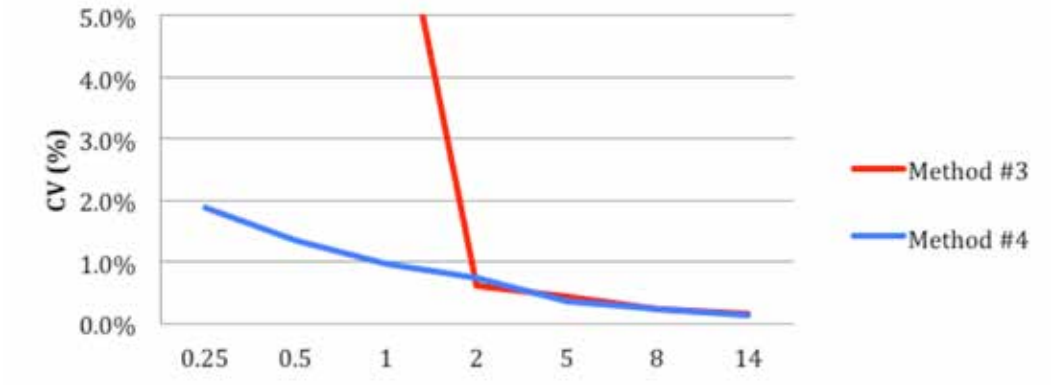


Figure 4 shows the advantage of over-aspirating liquid before carrying out the dispense. Both methods gave improved precision through the range above 2uL (versus Methods 1 or 2). However, only Method #4 allows one to dispense volumes all the way down to 250nL with excellent precision (CV < 2%)

Figure 5. ADP-Glo IC50 as a Function of Pipetting Method

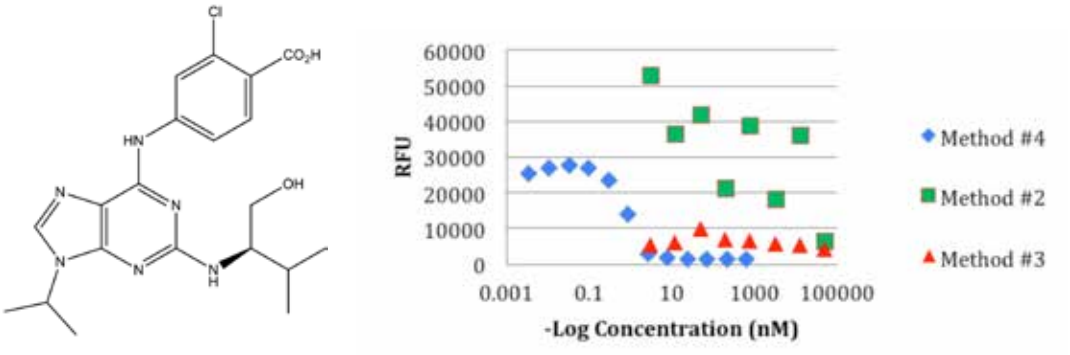


Figure 5 shows the dose-response curves obtained using CDK2 Inhibitor Purvalanol B in the ADP-Glo assay. 1uL of the inhibitor was added to the kinase reaction mixture using methods 2, 3 and 4. Only method #4 resulted in high quality dose-response curves. Note the extreme variation in amplitude in the Method #2 experiment. This is due to the large variation associated with the pre-aspirate/blow-off method. The generally reduced values observed in the Method #3 experiment is due to incomplete dispense of the liquid resulting from the "backlash" effect, described above.

Conclusions

- Attaching a manual multi-channel pipettor to a motorized framework increases liquid handling precision approximately one order of magnitude (20uL down to 2uL, and 2uL down to 250nL).
- Modifications of the basic aspirate/dispense steps can greatly affect accuracy and precision. The more air in the system, the lower the observed precision.
- An automated gravimetric method was developed to determine the precision and accuracy of the new platform.
- Application of this methodology to the ADP-Glo assay resulted in highly reproducible data.

About Us

Hudson Robotics, founded in 1983, has a long-standing history in robotic automation. Located inSpringfield, New Jersey, Hudson Robotics is a leader in microplate automation, laboratory robotics, liquid handling and customized software-driven solutions for life-science research. The company provides tools that can be used in areas such as drug discovery, clinical research and pharmaceutical development, including high throughput screening, proteomics and genomics.

We work with our clients to develop strategies that best meet their unique needs, whether for a large integrated system of automated laboratory equipment, or a single solution product that solves a specific problem.

Hudson Robotics is a privately held corporation with corporate headquarters and manufacturing inSpringfield, New Jersey. Sales, Service, and Marketing functions are also on the West Coast inCalifornia; Hudson has global representation through a dealer/distributor network that includes Europe, Asia, Australia and South America.