Technical Overview

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Angled Dispenser Tips for Reagent Addition to Live Cells

For use with Agilent BioTek Synergy H1, Synergy Neo, and Cytation 3 multimode readers

Introduction

Live cell-based assays have become ever more popular in microplates. While calcium flux assays have been the hallmark of G-protein coupled receptor assays for quite some time, recently high content phenotypic analysis of live cells is being used in drug discovery. As with homogeneous assays, a subset of cell-based assays requires the use of reagent dispensers that have been integrated into the microplate reader. There are numerous reasons to use automated reader dispensers in conjunction with a cell-based microplate reader.

The rapidity of response in some kinetic assays requires precise timing between the addition of a critical reagent and the determination of the results. Only with appropriate reader control of the reagent addition can the timing be evaluated at a high enough tolerance to ensure that all wells are treated the same. For example, calcium flux reactions often take place over the course of several seconds, which would preclude the addition of reagents manually.

Convenience is another reason to use automated reagent dispensers. The addition of reagent periodically over long time periods, laborious if added manually, can be automated with integrated reagent dispensers. This saves time and energy, while providing more consistent results.

Many microplate readers have temperature and CO_2 and/or O_2 gas control, and removing the plate from the reader would expose the cells to ambient temperature and atmospheric gas concentrations. The use of integrated dispensers can also avoid the exposure of the cells to environmental changes such as temperature or gas concentrations.

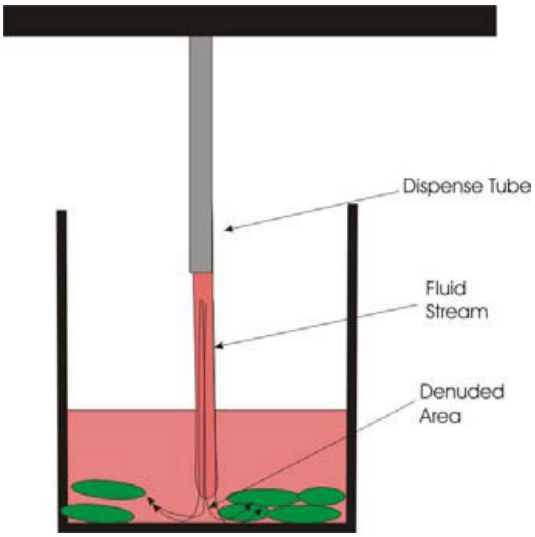

Figure 1. Schematic depiction of fluid flow with straight dispenser tips. Straight tips provide the best reagent mixing at the expense of potentially displacing adhered cells.

To get the most consistent results when using reagent dispensers, it is critical to rapidly mix the added reagent to the existing liquids within the microplate well. Volume, rate, and tip position are three important parameters in mixing. The volume of reagent being dispensed relative to the amount of fluid already in the well is probably the most influential parameter. More efficient mixing will occur when a greater percentage of reagents is added to the total volume (existing + fluid added). For example, adding 100 µL of (3 mM) solution to an existing 200 µL will mix more efficiently than if 5 µL of a 60 mM solution were added to 295 µL, even though the final concentration of reagent is the same (1 mM). The rate at which fluid is added can also affect the mixing efficiency, with faster fluid rates being more effective at mixing than slower rates. Reagent tip positioning can also play a role. The fluid entry angle into the well and the tip position relative to the well center are both important parameters. Centering the dispense tip on the well and keeping the tip vertically oriented provides the best mixing profile.

However, the high-efficiency mixing provided by straight tips can be deleterious for cell-based assays. The force and volume of fluid being injected into the well may be sufficient to displace cells adhered to tissue culture-treated microplates. This is of particular concern for microscopy applications, as the cells will no longer reside in the focal plane, and so will not be imaged. A good example of this phenomenon is demonstrated in Figure 2, where a NIH3T3 fibroblast monolayer was significantly impacted by a reagent dispense.

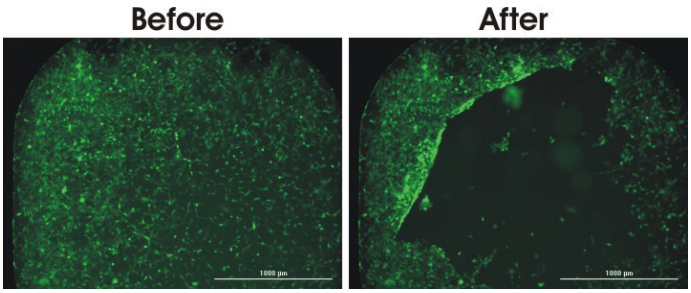

Figure 2. Effect of straight dispenser tip design on a cell monolayer. GFP expressing NIH3T3 cell monolayers in 384-well plates were subjected to a fluid dispense (25 µL of PBS) using a straight dispense tip. Wells were imaged at 4x before and after dispense using an Agilent BioTek Cytation 3 cell imaging multimode reader.

In this example, the force of the dispensed fluid flow was enough to overcome the dampening affects of the fluid already present in the well and scour away the cells from the microplate bottom, much like a fast flowing stream will move rocks on a stream bed. The liquid already present in the well normally serves as a buffer to slow the dispense fluid, but too little well fluid, or too much reagent, can overcome the shock-absorbing capacity of the well. While one might be able to alter the volume or dispense rate of fluid being added to avoid monolayer damage, the easiest solution is to use angled tips with live cell-based assays that require reagent addition within the reader.

By directing the flow sideways, fluid is no longer directed straight downward towards the cell monolayer, but rather through the fluid on top (Figure 3). This provides efficient reagent mixing without displacing adhered cells.

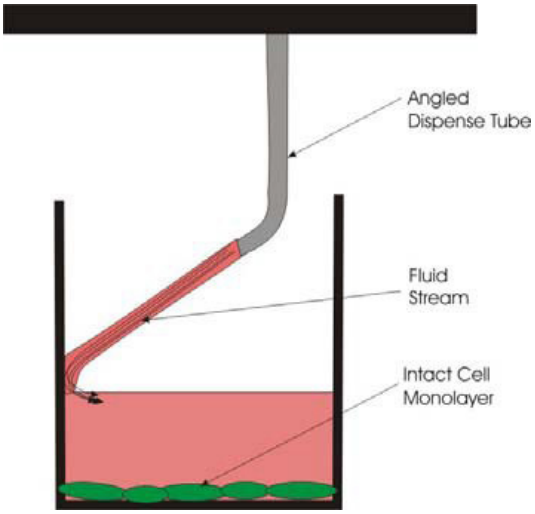

Figure 3. Schematic depiction of fluid flow with angled dispenser tips. Angled tips allow the dispensing of fluid against the side of the well, dissipating some of the fluid force and allowing cells to remain adhered.

Agilent has developed a dispenser tip option for the Agilent BioTek Synergy H1 multimode reader, Synergy Neo HTS multimode reader, and Cytation 3 cell imaging multimode reader to dispense reagents to live cells (Figure 4). The standard dispenser head uses stainless steel tubing fitted into an assembly that allows easy tool-free installation and removal from the reader. The optional dispenser head (part number 1320514) uses stainless tubes that have been angled 20° in the same quick change assembly. In addition, the dispenser head is offset so that the ends of the angled tips are positioned at the same spot above the well as the standard dispenser head tips. This feature enables the end user to use either dispense head interchangeably without having to worry about positioning or changing plate data files.

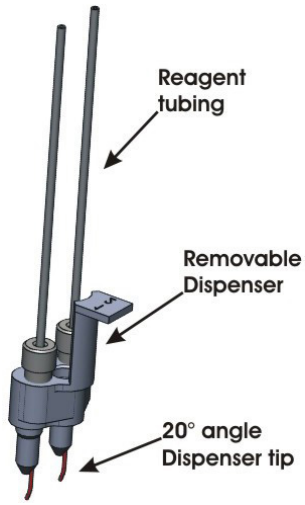

Figure 4. Angled tip design. Dispenser tips with a 20° angle allow fluid dispensing without displacing adhered cells. The changeable design allows switching between angled and straight tips, which work best with homogeneous assays.

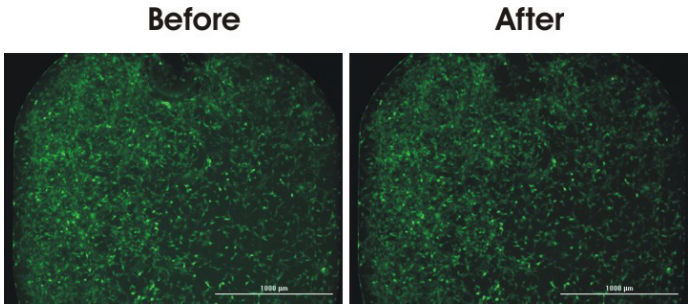

Figure 5. The effect of angled dispenser tip design on a cell monolayer. GFP expressing NIH3T3 cell monolayers in 384-well plates were subjected to a fluid dispense (25 µL PBS) using 20° angled dispense tips. Wells were imaged at 4x before and after dispense using an Agilent BioTek Cytation 3 cell imaging multimode reader.

The effectiveness of the angled design is demonstrated in Figure 5. NIH3T3 cells expressing GFP were grown to confluency in 384-well plates and subjected to a 25 µL fluid dispense. Representative images taken of the same well before and after reagent addition show virtually no damage to the cell monolayer. An example of the utility of the angled dispenser tips is their use in monitoring calcium flux with GPCR activation. CHO cells loaded with FluoForté dye (Enzo Biochem, Inc., Farmingdale, NY) demonstrate a rapid increase in fluorescence due to calcium flux upon stimulation with histamine (Figure 6). Ligand-induced G-protein coupled receptor (GPCR) activation is known to result in increased intracellular calcium ion levels. As free calcium ion levels increase, their interaction with the calcium-sensitive dye results in fluorescence intensity increases. The rapidity of the calcium change requires that the reader dispenser tips be used to add the stimuli.

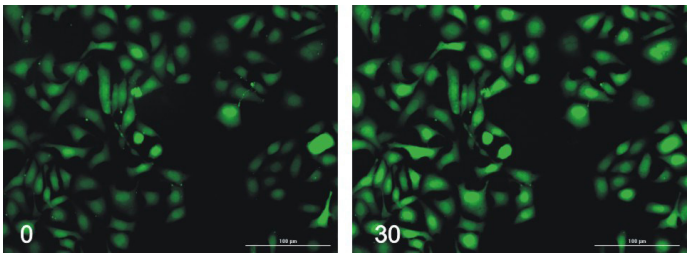

Figure 6. GPCR activation induced calcium flux. 20x images of CHO cells loaded with FluoForté before and after stimulation with 1 µM histamine and the fluorescence imaged. Time in seconds after stimulation is indicated in each image.

As stated previously, not all assays are the same when it comes to reagent addition. There are always compromises regarding reagent volume, concentration, and dispense rate with optimization. With homogeneous assays, the key element is typically adequate mixing without spilling or overflow of liquids inside the reader. Live cell-based assays have an added layer of complexity because the adhered cells need to be maintained within the context of adequate fluid mixing. The angled dispense tip is another tool that can be used for assay optimization.

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DE44334.2619560185

Printed in the USA, June 24, 2022
5994-3437EN

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