

The EL406™ Microplate Washer Dispenser Combines Thorough Washing and Precise Dispensing in One Space-Saving Instrument Configuration

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BioTek EL406

Abstract

Efficiency and productivity are increased with BioTek Instruments' new EL406™ Microplate Washer Dispenser. This automation friendly, multi-functional instrument uniquely combines the industry standard EL405™ Select CW Washer with a peristaltic reagent pumping system and two highly accurate syringe drive dispensers. The wash module uses BioTek Instruments' patented Dual-Action™ manifold design for a variety of microplate formats along with optimized flow rates for cell-based applications. The peristaltic pump utilizes a unique design to provide accurate and precise volumes ranging from 1 μ L to 10 mL. Three different autoclavable cassettes (1 μ L, 5 μ L, and 10 μ L) are each designed to deliver full incremental volumes for better performance. With two optional syringe pump dispensers, a total of three reagents can be dispensed without operator intervention. All functions are controlled by Microsoft® Windows® compatible software. Here we will demonstrate utility of this new design as applied to tissue culture cell experiments.

Introduction

The fluid handling demands of tissue culture experiments are both rigorous and variable, often requiring several different instruments to accomplish the necessary tasks. Seeding tissue culture cells into 96- or 384-well microplates requires several different capabilities. The fluid path must be sterile in order to prevent bacterial contamination. While sterile single-use devices have been employed, a reusable autoclavable fluid path is more desirable. Fluid dynamics, such as dispense rate and pressure, must be compatible with the viability of cells in suspension during the seeding process. Different cell types or experimental conditions often are performed on a single microplate, requiring the ability to dispense different solutions in different strips simultaneously. In addition, the ability to dispense different cell types, or not to dispense to rows or columns of cells, is a benefit in that it allows experimental flexibility. In regards to washing cells grown in a monolayer, the fluid dispense and aspiration rates need to be variable enough to provide adequate washing, but also gentle enough such that cells are not dislodged from the microwell surface. Fluid dispense rates that are too vigorous may denude the bottom of the well, particularly if the cells are loosely adherent. Rapid, accurate and precise addition of multiple reagents allows biochemical assays to be performed on the plated tissue culture cells. For example, cells can be lysed and assayed directly in the well using the BCA method of protein determination. The only requirement is that specific volumes of lysis reagent followed by working BCA reagent be added to the microplates.

The EL406 Washer Dispenser combines several existing fluid handling technologies into one instrument. The instrument builds on the industry standard design of the EL405 Select CW. The patented Dual-Action manifold provides full microplate washer functionality in both 96- and 384-well microplates. The priming trough has been replaced with an ultrasonic bath to provide easy cleaning of the aspiration and dispense tubes. Immediately adjacent to the full microplate washer manifold is an eight-channel peristaltic pump dispenser manifold. This 96- and 384-well microplate-capable dispenser uses autoclavable cassettes that come in different sizes to optimize fluid dispense accuracy and precision. In addition to the low dead volume, reagents can also be recovered by reversing the direction of the peristaltic pump. Two optional syringe pump dispensers can also be utilized to dispense additional reagents. Each of these two dispensers can rapidly dispense a single reagent to 96- or 384-well microplates.

Materials and Methods

Cell Washing

HEK293T cells were cultivated in DMEM (10% FCS) and plated into Costar 96- or 384-well microplates (Corning, Inc., Corning, NY) at 37°C. The following day, randomly selected wells were photographed using a Discovery-1 Screening System (Universal Imaging Corporation, Downingtown, PA) with 2x and 4x light transmission objectives. After taking the baseline image, microplates were then washed with PBS (3 wash cycles) using an EL406 programmed with a dispense rate of 2, which is optimal for loosely adherent HEK293T cells. After washing, the same wells were re-photographed to ascertain the utility of the cell wash programming. After determination that the cells were unchanged using the optimized washing protocol, the microplates were re-washed using rate 3, which had previously been found to work with strongly adherent cells, and photographed a third time. All photographic data was recorded using an integrated CCD camera saved as digital files that were collated using Adobe® Photoshop®.

Cell Seeding

Primary mesothelioma cells were cultivated in DMEM (10% FCS). Cell lines were trypsinized, counted and resuspended in fresh media at a density of 30,000 cells/mL. Using the peristaltic pump dispenser, cells were seeded into Corning 96-well plates (catalog number 3603) at 37°C. By leaving the A-channel tubing outside the cell suspension reservoir, only 7 of the 8 rows (rows B-H) had cells, while row A of the microplate was left empty. Row A was later utilized as locations for protein standards when the cells were assayed for protein content. On each of the three days following seeding, randomly selected wells were photographed using a Canon, Inc. digital camera attached to a Leica inverted microscope with 2x light transmission objective. All photographic data was recorded using an integrated CCD camera and saved as digital files. Each microplate was assayed for protein content using the BCA method.

Reagent Dispensing

Dispense accuracy and precision was determined using either a gravimetric method or the absorbance of dye solutions. Determinations using the gravimetric method were performed by weighing 8-well strips or entire microplates using a Sartorius LA120S analytical balance (Sartorius Corporation, Edgewood, NY). After dispensing fluid, the microplate and/or strip was quickly re-weighed. The resultant weight change, when divided by the number of wells, returns an average per-well dispense volume. When calculating the dispense precision, an aqueous solution containing FD&C yellow number 5 dye was dispensed into microplates with the EL406's syringe pump reagent dispenser. After mixing on an orbital shaker for 30 seconds, the absorbance at 450 nm (630 nm reference) was measured using a Synergy™ HT Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT). The %CVs were then calculated from the resultant absorbance values.

BCA Protein Assay

Cell culture uniformity was assessed using a BCA protein assay to quantitate total protein content in each well. The BCA protein assay performed as described in the instructions of the BCA-1 kit from Sigma-Aldrich (St. Louis, MO). The assay, essentially as described by Smith et al. (*Anal. Biochem.* 150:76-85) is based on the reduction of Cu(II) to Cu(I). The media was removed from the cell cultures and the cells washed 1x with PBS. Lysis buffer (25 μ L) was then added and the cells were lysed for 15 minutes. Lysis buffer consists of 50 mM Hepes, 250 mM NaCl, 5 mM EDTA, 0.1% NP-40, 1 μ g/mL Leupeptin, and 1 μ g/mL Aprotinin. After lysis, 200 μ L of working protein determination reagent was added to each well using the EL406's syringe pump dispenser. Working protein determination reagent was prepared immediately before use by mixing 1 volume of 4% copper(II) sulfate pentahydrate with 50 volumes of BCA solution. Assay samples were incubated for 60 minutes at 37°C and their absorbance at 562 nm was determined using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Winooski, VT). Unknown concentrations were determined by interpolating a linear curve generated from the results of known standards.

Conclusions

BioTek's EL406 Washer Dispenser is capable of multifunctional liquid handling

Compact design

Different modules provide different functionality

Washer Module

- Provides variable dispense rates for cell washing
- Capable of 96- and 384-well microplate washing
- Ultrasonic bath provides self-cleaning

Peristaltic Pump Dispenser Module

- Easily sterilized by autoclaving
- Replaceable dispense cassettes
- Low dead volume
- Reliable tissue culture cell seeding
- Can provide multiple fluids within a single dispense

Syringe Pump Dispenser Module

- Accurate and rapid dispense of fluids
- No recalibration required

Figure 1. BioTek EL406 Washer Dispenser.

Washer

The EL406 washer manifold uses the proven design of the EL405 Select CW Microplate Washer. It uses BioTek Instruments' patented manifold design to overcome the difficulties presented by high density microplates and allows virtually the same functionality to the 16x24 matrix 384-well microplates as is expected with the 8x12 formatted 96-well microplate (Figure 2). Dispense and aspiration manifolds are physically separated and are arranged on top of each other. The lower manifold (dispense) is constructed in such a manner as to allow the tube from the above manifold (aspiration) to pass through and enter the well of the microplate. In order for the dispense tube to be able to dispense fluid into a small well while the aspirate tube is removing fluid from the same well, as is the situation when overflow and bottom washing is performed, the dispense pipe is tilted from vertical. This allows for the dispense tube to be offset from the center of the well, providing room for the aspiration tube, yet still allowing the fluid jet to enter the well from the side. This canted design also has the added benefit of providing a swirling motion of the fluid which results in a more vigorous wash. In addition, the EL406 incorporates a dual fluid path and software control designed specifically to lower the dispense rate to the lowest possible flow, without affecting dispense accuracy and precision (Figure 3). When the low-flow rates are selected, the flow control valve directs all fluid movement through the "low flow line", which has the ability to restrict flow to very low rates. When standard rates are selected, the flow control valve opens and allows full fluid movement through the system.

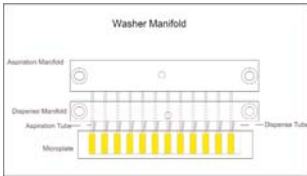


Figure 2. Schematic side view of the EL406's washer manifold.

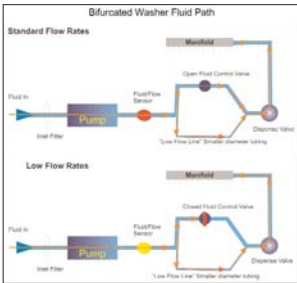


Figure 3. Bifurcated washer fluid path of the EL406.

Results

When an excessive fluid rate is used to wash loosely adherent cell lines such as human embryonic kidney (HEK293T) cells, a significant portion of the cells are disturbed. As demonstrated in Figure 4, when 293T cells in a 96-well microplate are washed with a non-cell wash setting, large portions of the well surface are observed to be denuded of cells after washing. This area generally was located to the left side of the well, which corresponds to the side toward which the PBS buffer fluid was dispensed. The same phenomenon is observed when 384-well plates are washed (data not shown). When the low flow rates are enabled, there is virtually no change in morphology when washing HEK293T cells. When the images from two different wells were obtained before and after washing with PBS for 3 cycles are compared, virtually no change is apparent (Figure 5). The cells remain attached and viable for further experimentation. Note that these two wells were found to be representative of the entire 96-well microplate.

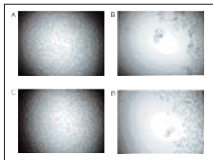


Figure 4. Before and after washing cells using standard dispense rate setting.

Two different wells of a 96-well microplate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2x objective.

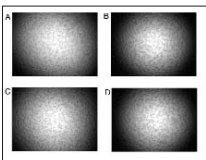


Figure 5. Before and after washing cells using low flow rate setting. Two different wells of a 96-well microplate before washing (A and C) and after 3 wash cycles (B and D) using PBS as the washer buffer. All images were obtained using the 2x objective.

Peristaltic Pump Dispenser

The peristaltic pump dispenser is compatible with 96- and 384-well microplates. It utilizes rollers that are fixed to a rotating rotor, which is encircled by stretched flexible tubing that has been compressed at the points where the tubing meets the rollers. As the rotor revolves, the fluid is forced to pass through the tubing. The volume that is pumped is dependent on the distance between the rollers, rotor position, tubing tension, inner diameter of the tubing and the geometry of the dispenser tip. By controlling these variables, accurate and precise liquid dispensing can be performed. BioTek uses a customized cassette design, which is optimized for volume and performance. Different tubing sizes (1 μ L, 5 μ L, and 10 μ L) are used to more closely match desired fluid volumes in full increments. These cassettes have eight individual tubes to deliver fluid. They are autoclavable to provide sterility, yet because of the materials used, do not require recalibration after such processes. The dispense head automatically makes adjustments for microplate height.

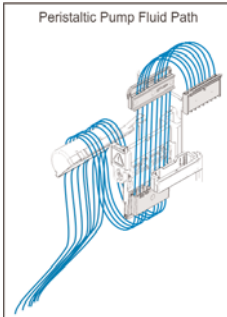


Figure 6. Diagram of the EL406's peristaltic dispenser pump fluid path.

Results

Figure 7 demonstrates the ability of the EL406's peristaltic pump dispenser to reliably seed tissue culture cells to 96-well microplates. These cultures were maintained at 37°C in a 5% CO₂ environment and remain sterile and viable for at least 72 hours after seeding. As indicated by the visible increase in cell density, these cells are actively growing and dividing cultures, suffering no harm from the method of seeding. Figure 8 demonstrates the uniformity and precision of cell seeding. When the optical densities of all wells of a microplate from a total protein determination are compared, very similar results are observed in all the wells of the microplate. The %CV of a BCA protein assay was found to be about 8% when measured 24 hours after seeding. Much of the variability is suspected to be the result of differential clonal growth of the primary tumor cells.



Figure 7. Primary mesothelioma cells seeded using the EL406's peristaltic pump dispenser.

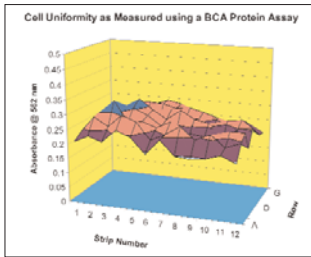


Figure 8. Uniformity of cell dispense using the EL406's peristaltic pump dispenser. Mesothelioma cells were seeded using the peristaltic pump dispenser to all the wells of a 96-well microplate in 200 μ L of media. Cell content was measured 24 hours after seeding using a BCA protein assay.

Syringe Pump Dispenser

The EL406's syringe pump dispenser is compatible with both conventional 96- and 384-well microplates. The microprocessor controlled syringe pump is based on a tested, low maintenance design that requires no recalibration, yet provides a high degree of accuracy and precision. Using unidirectional check valves to control fluid direction, the syringe dispenser draws fluid from a non-pressurized reservoir into 96- or 384-well microplates (Figure 9). As part of the manufacturing process, BioTek performs a 6-point calibration procedure to accurately tune the syringe pump over a wide range of volumes. Among the many variables which programming allows is the control of flow rates from a very low speed for dispensing to cell cultures to very rapid rates for vigorous reagent dispensing.

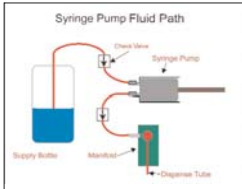


Figure 9. Schematic view of the EL406's syringe pump dispenser.

Results

As demonstrated in Table 1, the EL406's syringe pump dispensers are accurate across the entire range of its volume settings. When the minimum setting (10 μ L) was selected, the dispense-volume, determined gravimetrically, was found to deviate from the expected rate by 11.7% (8.8 μ L vs. 10.0 μ L). The deviation rapidly diminished with larger volumes to less than 1%. In all cases, the dispense volume was quite precise. As demonstrated in Figure 10, the coefficient of variance (%CV) was found to be less than 7% at 10 μ L per well. The %CV also decreased rapidly with larger volumes to less than 1% at volumes above 50 μ L per well.

Using the syringe pump dispenser to provide the working reagent for the BCA assay several different concentrations of a growth inhibitor were tested for their ability to inhibit cell growth. As demonstrated in Figure 11, a syringe dispenser reliably added a working BCA reagent to all the wells of the microplate. After color development at 37°C for 60 minutes, the absorbance was measured. Samples that had been treated with inhibitor showed a decrease in total protein content as compared with the untreated controls. These data demonstrate that not only can the syringe dispenser provide accurate amounts of assay reagent, but also that the peristaltic pump has provided equivalent amounts of cells when they were originally seeded.

Dispense Accuracy of the EL406's Syringe Pump Dispenser into 96-Well Microplates			
Expected Volume (μ L)	Calculated Volume (μ L)	% Deviation	
10	8.8 \pm 0.58	11.7	
20	18.5 \pm 0.99	7.6	
30	30.0 \pm 0.52	0.0	
40	39.5 \pm 1.21	1.2	
50	49.5 \pm 0.12	0.9	
100	99.4 \pm 0.05	0.7	
150	149.2 \pm 0.08	0.5	
200	198.8 \pm 0.15	0.6	
250	248.7 \pm 0.08	0.5	
300	298.0 \pm 0.68	0.7	

Note that these data represent the mean and average of twelve determinations.

Table 2. Dispense accuracy into 96-well microplates. Individual 8-well microplate strips were weighed. After the syringe pump dispenser had dispensed the indicated volume of fluid into each well of 12 strips (96 wells), the strips were re-weighed. The volume of each well was calculated by dividing the change of weight by the number of wells (8) in a strip. The data represents the average of 12 different 8-well strip determinations. The percent deviation is the ratio of the difference between the calculated and expected values to the expected values.

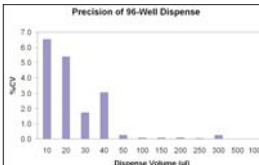


Figure 10. Dispense precision into 96-well plates using the EL406's syringe pump reagent dispenser at various dispense volumes.

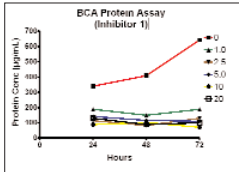


Figure 11. Representative inhibitor assay. Several different concentrations of inhibitor were assayed for protein content 24, 48, and 72 hours after seeding with the EL406.