Automated ELISA Liquid Handling with EL406 Combination Microplate Washer Dispenser

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BioTek EL406

Abstract

With a typical ELISA protocol, several repeat cycles of microplate washing, reagent addition and incubation are executed to remove unbound material before data collection. This often manual process requires a technician to manage timings and be available to move the plate between washer and multiple dispensers. The new EL406 Microplate Washer Dispenser increases a laboratory's efficiency and productivity by eliminating these manual plate movements. This automation friendly, multifunctional instrument uniquely combines the industry standard ELx405 Select CW Washer with a peristaltic reagent pumping system and two highly accurate syringe drive dispensers. The wash module incorporates BioTek's patented Dual-Action™ manifold design for both 96- and 384-well microplates 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 µL to 3 mL. With two optional syringe pump dispensers, a total of three reagents can be dispensed without operator intervention. Additionally, up to four reagent buffers can be used automatically. Together with a BioStack Microplate Stacker, as a batch processor the EL406 will manage all liquid handling steps of an ELISA inclusive of incubations. Users simply press a button and walk away eliminating the need for multiple instruments and saving both bench space and cost. This poster will detail specifics of such a system.

Figure 1. EL406 Microplate Washer Dispenser

ELISA Automation

Many biochemical assays involve the addition of different reagent fluids interrupted by delays or incubations to allow for a reaction to occur. Originally these assays were performed in test tubes, but they have since been converted to microplates as a means to reduce reagent volume and increase throughput.

ELISAs are ubiquitous in biomedical research and clinical testing. This heterogeneous assay typically uses a microplate as a solid surface to which specific antibodies are bound allowing the capture and analysis of a seemingly infinite number of analytes. It does so by a series of incubations, reagent additions and wash-steps. During incubations, specific molecules are bound to the solid substrate by interactions with the previously absorbed antibodies. Following the binding step, unbound materials are removed by the washing step. After the unbound material has been removed, the next reagent (usually a conjugate) is added comprised of a specific antibody and an enzyme covalently linked. Again unbound material is removed and the next reagent (substrate) added afterward. The substrate interacts with the enzymatic portion of the conjugate to produce a colored compound, which can be detected by absorbance, fluorescence or luminescence depending on the substrate used. Regardless of the analyte, the same process steps are used in ELISA.

The EL406 Washer Dispenser is an automated microplate processor that can perform microplate washing steps in 96- and 384-well microplates. In addition to standard wash routines, the EL406 has built-in cell-washing capabilities. A buffer switching valve allows for up to four different wash buffers to be selected without changing bottles. A built-in ultrasonic cleaner allows for automated maintenance of the wash manifold. In addition the device has up to three different reagent dispenser heads. One head is a peristaltic pump device capable of dispensing from 1 µL to 3000 µL. The EL406 can also be configured with an additional module that has two independent syringe pump dispenser heads capable of delivering from 5 µL to 3000 µL. The EL406 is capable of plate shaking at three different speeds and is robotic compatible.

The BioStack is a microplate handling and storage device capable of holding up to 50 plates. It uses a plate gripper to lift the microplate from the diving board of the BioStack and place it on the carrier of the EL406. The BioStack sequentially delivers plates from the bottom of the input stack to the EL406 and then receiving them into the bottom of the output stack. The net result is that the first plate to be processed will come from the bottom of the input stack and end up at the top of the output stack. Prior to subsequent processing, the stacks are moved from the output stack back to the input stack - a process called re-racking. Besides resetting the plates to be processed, this action also reorders them such that the first plate to process is again on the bottom of the stack.
By using the BioStack as both an input/output storage device and an incubation location for the EL406, heterogeneous assays that involve the addition of reagents to and the washing of microplates can be automated. Liquid Handling Control PC Software (LHC) can be used to control both the BioStack and the EL406 and coordinate their actions. This allows for the automation of many assays that involve the addition and removal of fluids at timed intervals. Here we describe the automation of the liquid handling portion of three different assays.

Avian Influenza Virus Antibody ELISA

Introduction

Avian Influenza Virus (AIV) is a viral disease of domestic and wild birds, which has a range of responses from almost asymptomatic to very high mortality. The Synbiotics ELISA kit is a specific screening test for the detection of antibody to AIV in chicken serum samples. The basis of the test is that serum from chickens exposed to AIV antigens will contain specific anti-AIV antibodies, which can then be captured on a test plate coated with AIV antigens through an antigen-antibody complex.

The screening of chicken flocks for Avian Influenza Virus provides an important role in ensuring that bird flocks are disease free. The sheer numbers of animals that need to be tested under normal circumstances in many animal test facilities requires large numbers of samples be processed daily. In the event of a disease outbreak, the test-volume would be expected to increase many fold above the current levels in specific regions. The ability to automate the process represents a tremendous savings in time, manpower, and cost. Here we describe the BioTek robotic system used to automate the liquid handling steps of the Synbiotics Avian Influenza Virus Antibody Test Kit.

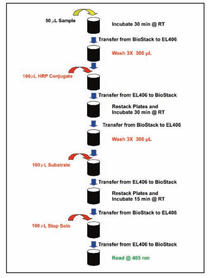

Figure 2. Avian Influenza Virus Antibody Assay Test Procedure Steps. The avian influenza antibody test used was an ELISA kit from Synbiotics and performed as described by the kit instructions. The absorbance of the wells at 405 nm was determined using a Synergy 4 Multi-Mode Microplate Reader with Hybrid Technology (BioTek Instruments). Processes carried out by the EL406 Microplate Washer Dispenser are indicated in Red.

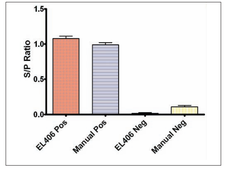

Figure 3. Comparison of Manual and Semi-automated EL406 Processing. Positive and negative kit controls were treated as unknown samples and the signal to positive control ratio (S/P) was calculated. Data represents a total of 72 samples of each from 3 different assay plates.

Results

- **Semi-automation of Avian Influenza Virus Antibody detection assay is achieved using the EL406 Washer Dispenser.**
- **Plate movement and processing are controlled using Liquid Handling Control (LHC) Software.**
- **Very similar S/P Ratios are observed between manual and EL406 processing.**
- **Well to well variability is similar in manual and semi-automated EL406 processing.**

Melamine Quantitation ELISA

Introduction

Melamine is a nitrogen rich compound normally used as either a flame retardant or in conjunction with formaldehyde to produce melamine resin, a durable thermosetting plastic used in the manufacture of countertops, fabrics, and glues. However, in addition to the normal use of melamine, several illicit uses for the material have been reported. The practice of using melamine scrap as an additive to animal feed and food products to give the appearance of increased protein content is widespread in many countries. Recently a scandal in China has implicated over two dozen companies and numerous individuals of adding melamine to milk and infant formula, leading to kidney stones and renal failure, and resulting in the deaths of several infants while sickening over 53,000 others.

The melamine assay is a competitive ELISA, where melamine-HRP conjugate competes for binding to the melamine antibody attached to the wells of the microplate. Following the completion of the binding reaction, unbound sample and conjugate is removed by washing. Substrate reagent is immediately added and the color allowed to develop. The color-development reaction is terminated by the addition of stop solution, and the absorbance of each well is determined. Unknown concentrations are then determined by interpolating a standard curve generated by running standards of known melamine concentrations. In order to easily compare multiple experiments, the data is expressed as a ratio to the zero-standard. This ratio is often expressed as B/Bo. Here we describe the use of an EL406 Washer Dispenser in conjunction with the BioStack and LHC Software to semi-automate this ELISA application.

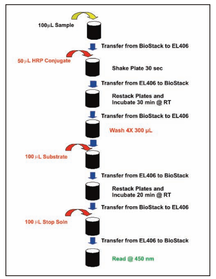

Figure 4. Schematic diagram of the procedural steps of the melamine ELISA reaction. The melamine assay used was an ELISA kit from Abraxis and performed as described by the kit instructions. The absorbance of each well at 450 nm was determined using a Synergy 4 Multi-Mode Microplate Reader (BioTek Instruments). Processes carried out by the EL406 Microplate Washer Dispenser are indicated in Red.

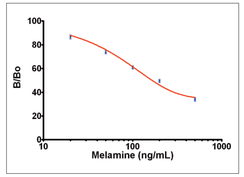

Figure 5. Melamine Concentration Curve.

Results

- **Competitive assay demonstrates decreasing signal with increasing melamine concentration.**
- **B/Bo calculation is described by a 5-parameter logistic fit.**
- **ELISA results are very similar to data reported with kit insert.**
- **Detection limit range from 10-30 µg/mL agrees with kit insert data.**

EL406 Saves Space and Time

Introduction

Laboratory bench space is a valuable asset that is often in short supply. Physically large instruments or the requirement for multiple instruments to perform assay functions leads to a crowded and cluttered work area that decreases productivity. Because the cost of adding new laboratory space is often cost-prohibitive, in order to improve productivity it is necessary to develop instrumentation that provides multiple functionalities in a compact package. The EL406 Microplate Washer Dispenser provides the same functionality as four individual instruments (ELx405 Washer, 2 MicroFill and 1 MicroFlo Select Dispensers), in a footprint only slightly larger than that of the ELx405 washer it replaces (Figure 6). Even when a BioStack plate storage device is included with an EL406, the footprint is considerably smaller than the combined footprint of four instruments. In addition to reducing footprint, the use of the EL406 Microplate Washer Dispenser will reduce assay times by eliminating the need to move plates between dispensers and washers.

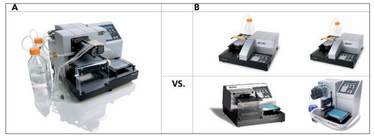

Figure 6. Multi-component manual assay and EL406 semi-automated systems. Figure A depicts an EL406 Washer Dispenser. Figure B depicts the four instruments (ELx405 Washer, MicroFlo Select Dispenser, and 2 Micro-Fill Dispensers) necessary to provide the equivalent capability of the EL406 Washer Dispenser.

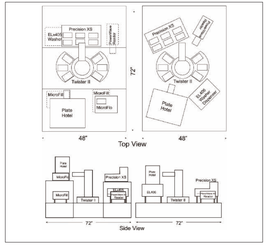

Figure 7. Schematic diagram of an Automated ELISA Twister II system with and without an EL406 Washer Dispenser. The necessary equipment to completely automate the Avian Influenza virus test kit process has been depicted using either individual components (A) or with an EL406 Washer Dispenser (B).

Table 1. Simulated Assay Times for Synbiotics Avian Influenza Virus Antibody Test Kit

Plate #	EL406 System	Component System
1	1:18:30	1:19:45
5	1:24:30	1:28:45
10	1:32:00	2:27:15
15	2:26:30	2:42:00
20	2:34:00	3:38:15
25	3:28:30	4:35:00
30	3:36:00	4:52:30
35	4:30:00	5:50:00
40	4:38:30	6:05:15

Results

- **EL406 Washer Dispenser provides the same functionality as four individual instruments.**
- **Robotic systems that utilize the EL406 rather than multiple instruments are less complex and require significantly less space.**
- **Standard lab bench is utilized rather than an expensive custom table.**
- **Cabling and access to reagent reservoirs is much easier with a single tier set up.**
- **Using an EL406 in lieu of individual components saves considerable amounts of time by eliminating movement steps.**

EL406 with BioStack

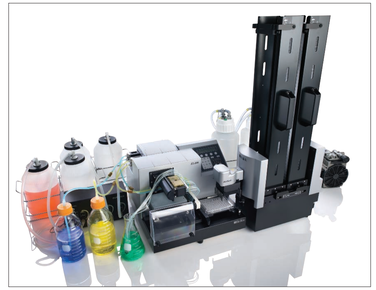

Figure 8. EL406 with Integrated BioStack.

Conclusions:

EL406 provides equivalent results as compared to manual processes.

EL406 semi-automates ELISA and heterogeneous wash assays in general.

EL406 saves bench space and reduces complexity in automated robotic systems.

EL406 saves time by eliminating plate movement in automated robotic systems.

EL406 offers a significant cost savings compared to separate instruments – 1 washer and 3 dispensers.