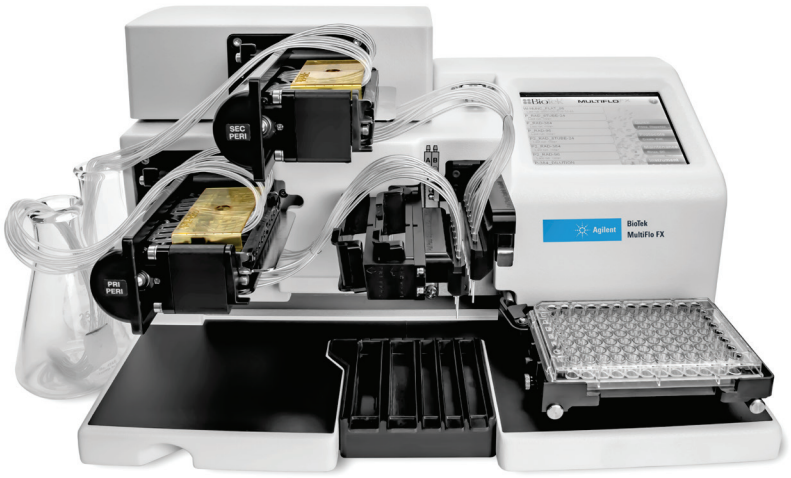
Agilent

Trusted Answers

Optimization of Microplate Washer Performance

Application Compendium



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Introduction

The utility of microplate washers

Microplates are the laboratory sample vessel of choice when performing an array of assay types where many samples need to be analyzed, or statistical data is required. Historically, microplate assays are run in a 96-well format, however both higher and lower densities are available, such as 6-, 12-, 24-, 48-, 384-, and 1536-well formats. Regardless of the type or density of microplate used, every assay will require several discrete additions, including both sample and reagents. Often the assay workflow requires removal of reagent added in excess to drive reaction kinetics or unbound sample matrix. Without this removal, assay background would be unacceptable. This is typically performed by several aspiration and dispense cycles of wash buffer. Especially when using higher-density microplates, the removal from and addition to the wells quickly becomes tedious and error prone if performed manually with handheld pipettors.

Microplate washers are designed to automate this process, leading to rapid microplate processing times and improvement of assay precision relative to manual washing. BioTek Instruments, now a part of Agilent Technologies, Inc., has a rich history in developing microplate washers that spans over 40 years. Its first microplate washer, EL402, was designed and manufactured for ELISA assays in 1981. Since that time, Agilent BioTek has incorporated multiple technologies into the current microplate washer product line. These will be described in this application compendium.

Agilent BioTek microplate washer-based technologies

Dual-Action manifold

The Agilent BioTek Dual-Action manifold design allows independent control and positioning of both the aspirate and dispense sections of the wash manifold, providing major benefits, such as:

- Rapid washing of either 96- or 384-well plates on one platform
- Ideal positioning of dispense tubes for standard washing, in addition to gentle washing of sensitive cell-based assays
- Optimization of aspirate tubes to allow more or less residual after dispense, as well as cell layer removal prevention
- Overfill and overflow washing with continuous fill and aspiration at the tops of the wells

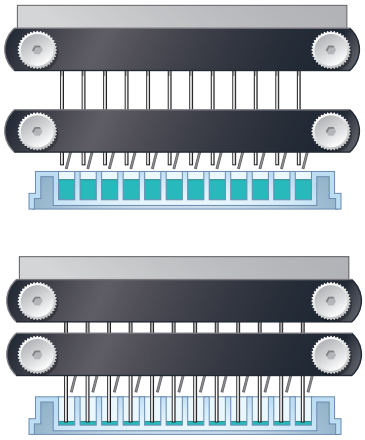


Figure 1. the Agilent BioTek Dual-Action manifold allows independent control of dispense and aspirate tubes for optimal X-, Y-, and Z-positions. Dispense tubes are angled to 20° for gentle dispensing into wells containing delicate cell layers.

The following Agilent BioTek instruments incorporate the Dual-Action manifold in standard or optional configurations: 405 Touch washer, EL406 washer dispenser, MultiFlo FX multimode dispenser, and ELx50 strip plate washer.

Verify

Unobstructed fluid paths in liquid handling instruments are critical to the optimal performance of the instrument. Microplate washers are especially susceptible to partially or fully clogged dispense and aspirate tubes from salt and protein buildup, even when routine maintenance is performed. Frequently, blocked wells go unnoticed until the assay fails and the data is rendered inaccurate, costing time and money. The Agilent BioTek Verify technology is a built-in module that tests the performance of washer dispense and aspiration cycles, and alerts users to the specific location of any suspected clog. This allows the blockage to be identified and resolved before the next assay is run. The ability to check for blockages prior to a run is ideal not only for benchtop applications, but especially when the washer is integrated into a large automated system—where blockages can cause significant downtime.

Figure 2. (A) The Agilent BioTek Verify technology is built in above the manifold. The Verify probe (B) sends ultrasonic waves into a microplate to determine the fluid volumes in each well following dispense and aspirate cycles.

The Agilent BioTek microplate washers, equipped with Verify clog detection technology, include a preprogrammed quality control (QC) routine for periodic testing of each individual dispense and aspiration tube's function. The QC protocol fills microplate wells and confirms their fluid levels using an integrated distance sensor. Microplate wells are then aspirated, and again the fluid levels are measured to confirm fluid has been evacuated. Results are displayed on the 405 TS screen or in the Agilent BioTek Liquid Handling Control (LHC) software. Based on the pass or fail result, the user can clean the manifold, using the proprietary Agilent BioTek Ultrasonic Advantage manifold cleaning system. Verify technology can be run a second time to ensure the blockage is cleared and the washer is ready for use.

Angled dispensing

As cell-based assays become more prevalent in research, the benefits of angled tubes become increasingly important. The angled dispense tubes can be positioned to provide a gentle flow of medium, buffer, or reagent down the sides of a 96- or 384-well plate so as not to disrupt the cell layer (Figures 1 and 3).

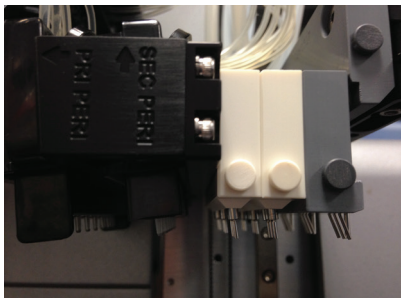


Figure 3. Agilent BioTek MultiFlo FX multimode dispenser angled tip configuration for peristaltic and syringe pump dispensers, as well as wash module dispense tubes.

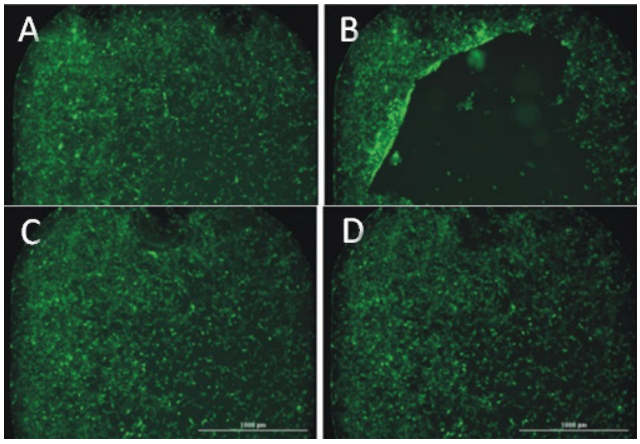


Figure 4. Effect of straight and angled tube dispensing onto cell layers. GFP-expressing NIH3T3 cell monolayers (A and C) in 384-well plates subjected to a 25 µL PBS dispense using (B) straight or (D) angled tubes.

Angled dispensing is available on the following Agilent BioTek instrumentation: 405 microplate washer, EL406 washer dispenser, MultiFlo FX multimode dispenser, and 50 TS washer.

Ultrasonic Advantage

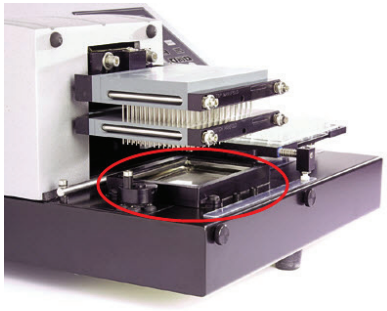


Figure 5. Priming/cleaning reservoir (circled) in the Agilent BioTek 405 washer containing Agilent BioTek Ultrasonic Advantage.

Poor microplate washer maintenance can lead to assay failure, system downtime, and increased costs. Unexpected clogging of washer manifold tubes is the single largest contributor to failures. However, cleaning can be a time-consuming and labor-intensive process. The 405 washers and EL406 washer dispenser can be configured with an available built-in ultrasonic cleaner to aid in routine washer maintenance. The proprietary Agilent BioTek Ultrasonic Advantage makes the 405 and EL406 the only self-maintaining microplate washers available today, eliminating washer-associated failure and guaranteeing continued washer performance.

Automated Media Exchange

Three-dimensional (3D) spheroidal cell models have become a mainstay in life science research due to the ability to mimic in vivo–like environments. Performing media exchanges and washes with spheroids in cell-repellent microplates can be problematic due to the risk of accidental spheroid removal. By incorporating the Agilent BioTek Automated Media Exchange (AMX) peristaltic pump–based tool on the MultiFlo FX multimode dispenser, these procedures can be carried out in a controlled manner that eliminates spheroid disruption and removal, enabling long-term 3D experimental procedures requiring multiple media exchanges. The proprietary AMX module consists of two unique, modified peristaltic pump cassettes with eight stainless steel tube aspirate (Figure 6A) and dispense (Figure 6B) heads. The cassette tubing is fed through the peristaltic pumps of the MultiFlo FX and into media bottles or tubes (Figure 6C). Software allows the pumps to run slowly and gently, and also enables the tubes to be moved to the side of a well, to avoid disturbing the spheroids during aspirate or dispense procedures. Each cassette is fully autoclavable, enabling sterile processing.

Figure 6. Sterile peristaltic pump cassette processing using the Agilent BioTek Automated Media Exchange module. Eight stainless steel tube aspirate (A); dispense heads (B); and Agilent BioTek MultiFlo FX multimode dispenser (C).

Common Assays Using Microplate Washers

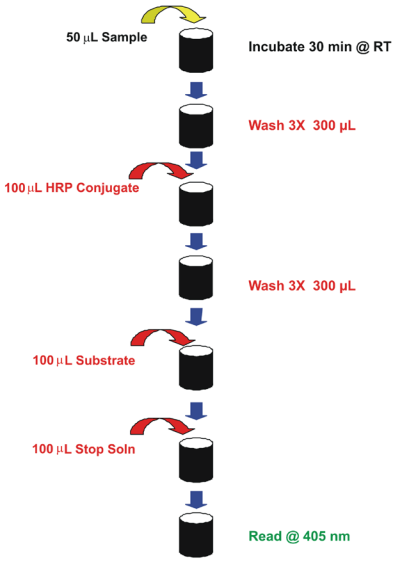


Figure 7. ELISA workflow schematic. The typical ELISA format requires a capture antibody that is specific for the antigen of interest, immobilized on a solid support (microplate). The sample is loaded into each well and bound. A series of wash steps are performed between reagent additions. An antibody-conjugate is applied over the surface, where it binds to the antigen. The antibody-conjugate is typically linked to an enzyme. An enzyme substrate is added and the subsequent reaction produces a detection signal. Signal generation is typically ended with addition of a stop solution.

Ultrasonic cleaning involves the rapid formation and violent collapse of minute vacuum bubbles that implode on surface contact. This agitation by countless small and intense imploding bubbles creates a highly effective scrubbing of both the inside and outside of the washer manifold tubes. Cavitation, as this process is called, is produced by the Ultrasonic Advantage technology, which automatically pulses the ultrasonic energy into a liquid-filled built-in cleaning reservoir in which the washer's manifold tubes are fully submerged. Even heavy buildup from protein and salt crystals is dissolved from within the tubes.

ELISA

ELISA workflow

The enzyme-linked immunosorption assay (ELISA) is a common procedure used in biomedical research, clinical testing and industrial manufacturing for both diagnostics and quality assurance. The assay relies on the use of antibodies, with the detection signal commonly being a change in color or production of a fluorescent signal. One advantage of performing an ELISA is the ability to isolate and detect a substance or antigen in a complex sample. The assay has been widely adopted for use in a microplate format for increased throughput. The method involves a series of reagent additions and wash steps to remove unbound material and excess reagents from the well as demonstrated in the standard workflow schematic shown in Figure 7. The introduction of automated plate washers and combination washer dispensers has resulted in both increased throughput and improved assay performance.

Factors affecting microplate washing

The wash step contains two distinct steps; aspiration of liquid and dispensing of wash solution to each well. A major cause of poor inter-assay precision stems from aspiration issues. Automated settings need to be optimized for each plate type during assay development. Parameters requiring optimization include:
Aspiration tube location:

- Flat-bottom plates—near the outer edge of the well where liquid accumulates
- Round-bottom plates—near the center of the well
- Minimize residual fluid (note: tubes should not touch the bottom of the well)

Vacuum pressure setting:

- Sufficient to effectively remove all fluid even during overfill dispense
- Not so high as to allow air to be drawn across absorbed protein coating
- Avoid high vacuum pressure, which can result in shear stress
- Avoid complete evacuation of well, which can result in protein denaturation

Soak and shake:

- Used for improved removal of nonspecific bound material
- Duration and intensity can be optimized

While each assay will require optimization depending on the plate type and reagents used, the tables below provide an example of the recommended parameters for performing an ELISA in a 96-well, flat-bottom plate (Tables 1 to 3).

Table 1. Agilent BioTek 405 washer. Wash *method* parameters for 96-well, flat-bottom plate.

Number of Cycles	3 to 5
Soak/Shake	Most do not soak.
Soak Duration	If used, 5 to 30 s.
Shake Before Soak	Most do not shake.
Shake Duration	If used, 5 to 15 s.
Shake Intensity	Any intensity would work—faster settings provide more vigorous mixing.
Prime After Soak	Generally not necessary, unless a very long soak time.
Prime Volume	If used, 200 mL.
Prime Flow Rate	If used, 5 to 7.

Table 2. Agilent BioTek 405 washer. Wash *dispense* parameters for 96-well, flat-bottom plate.

Dispense Volume	300 to 375 µL; if shaking, 200 to 250 µL to prevent splashing
Dispense Flow Rate	3–5 or 7–9 for assays requiring more intense washing
Dispense Height	114–116; 120 absolute highest. Settings of ≤ 114 could limit volume remaining after dispense
Horizontal Dispense Position	00 setting. Typically works well with 96-well flat-bottom plates
Bottom Wash First	Normally not used; if used, 50 height, 300 µL volume and 5–7 flow rate
Prime Before Start	Normally not used; if used, this would prime before every wash program

Table 3. Agilent BioTek 405 washer. Wash *aspirate* parameters for 96-well, flat-bottom plate.

Aspirate Height	27–28, depending on plate height. Settings may hit well bottom
Horizontal Aspiration Position	–25 to –30. Aspirate tubes should be 1–1.5 mm from left well edge. A 00 setting will leave ring with flat bottom
Aspiration Rate	3–5; 5 useful for buffer with no surfactant (e.g., Tween)
Aspirate Delay	If used, 0500 ms; 0750 ms useful for buffer with no surfactant (e.g., Tween)
Crosswise Aspiration	Yes, sometimes not necessary on every cycle
Crosswise Aspiration on Final	Yes
Horizontal Aspiration Position on Crosswise	10. Dispense tube should be 1–1.5 mm from right well edge
Final Aspiration	Yes, if the assay requires
Final Aspiration Delay	If used, 0500 ms

Avian influenza virus antibody ELISA

The avian influenza virus (AIV) is a viral disease of domestic and wild birds, which has a range of responses from almost asymptomatic to high mortality. Synbiotics offers an AIV 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. Because of the sheer number of animals that need to be tested under normal circumstances in many animal testing facilities, large numbers of samples need to be processed daily. The EL406 washer dispenser and the Agilent BioTek BioStack microplate stacker have previously been used to automate washing and dispense processes, as demonstrated in the workflow schematic in Figure 8. Washing and dispense parameters were optimized based on recommendations provided in Tables 1 to 3.

The experimental data show similar results between manual and automated methods (Figure 9).

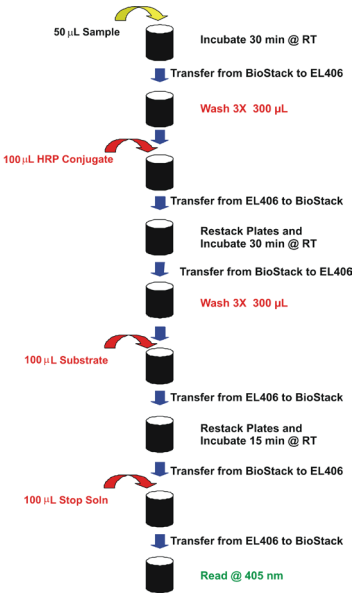


Figure 8. Avian influenza virus antibody assay test procedure workflow schematic. Processes carried out by the Agilent BioTek EL406 washer dispenser are indicated in red and the Agilent BioTek BioStack microplate stacker in black text.

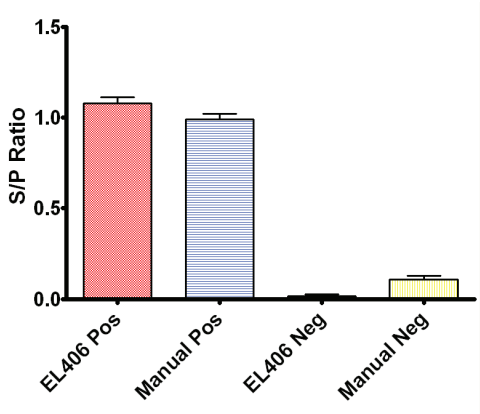


Figure 9. Comparison of manual and semi-automated processing with the Agilent BioTek EL406 washer dispenser. Positive (+) and negative (-) kit controls were treated as unknown samples, and the signal-to-positive control ratio (S/P) was calculated. Data represent a total of 72 samples of each from three different assay plates.

Solution ELISA assay for immunogenicity testing of biological drug products

Several challenges have surfaced during clinical evaluation of biological drug products due to a commonly associated immune response in patients. Anti-drug antibodies (ADA) are known to be frequently generated during administration of humanized monoclonal antibody therapeutics. These ADAs are nearly indistinguishable from antibody drug therapeutics, and require robust selective methods to determine the extent to which they impact safety and efficacy during treatment. A commonly used technology platform for assessment of immunogenicity relies on the bridging immunogenicity assay format typical of ELISA. The solution ELISA also relies on bivalent binding of antidrug antibodies to biotinylated- and digoxigenin-labeled drugs. Upon complex formation, the complex is captured on a streptavidin-coated microplate. Assay quantification is accomplished by complex identification by an anti-digoxigenin monoclonal antibody HRP conjugate and subsequent measurement of chemiluminescent signal intensity. As this assay is typically performed on a small sample size, a strip washer can be used to automate washing procedures in combination with the MultiFlo FX for reagent dispensing, as demonstrated in the workflow shown in Figure 10. Washing and dispense parameters were optimized based on those recommendations provided in Tables 1 to 3.

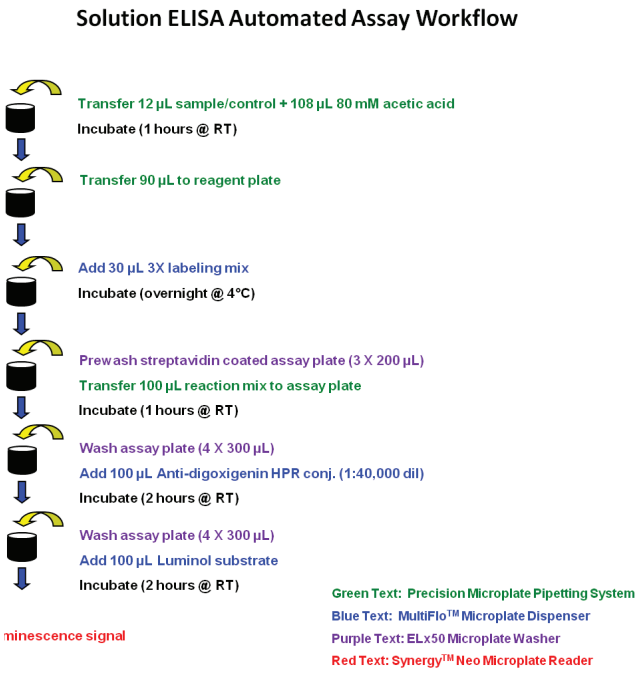


Figure 10. Solution ELISA automated assay procedure. Plate washing was accomplished by Agilent BioTek ELx50 washer shown in purple text.

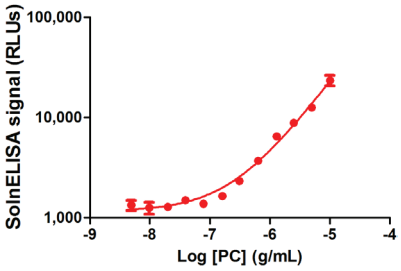


Figure 11. Assay sensitivity, assessed by performing an 11-point 1:2 serial dilution of the positive control starting at a concentration of 10x the HPC plus a zero concentration point.

Figure 11 demonstrates a dilution series of the positive control for the assay. The use of automated methods results in excellent precision as seen by the low standard deviation of replicate data points even at low positive control concentrations.

Luminex xMAP

Assay principle Luminex xMAP workflow

Bead-based, multiplexing assays such as those provided by the Luminex xMAP technology (Luminex Corporation; Austin, TX), have proven to be useful for biomarker identification and quantification from cells, tissues, and body fluids. Applications include biological research, drug discovery, and clinical diagnostics. The original technology based on the use of polystyrene microspheres tends to have arduous, manual workflows that are prone to cumulative systematic and random errors that can lead to poor precision for quantification. Agilent BioTek microplate washers are capable of processing both polystyrene and the more recently developed magnetic microspheres allowing automation of workflows in both partially full microplates or full 96- and 384-well microplate formats.

The original xMAP technology is based on the use of polystyrene microspheres. Microplate-based sample preparation uses vacuum filtration to remove sample matrix and excess reagents in a fashion similar to cell membrane–based receptor–ligand binding experiments. While acceptable analytical performance can be achieved, caution must be taken to prevent clogged wells, and automation can be difficult. More recently, available magnetic microspheres have significantly improved ease of use and make the xMAP technology much more automation friendly. MagPlex microspheres (Luminex Corporation; Austin, TX) can be washed by either vacuum filtration or, preferably, by use of a custom magnet for Agilent BioTek washers with either flat or ring designs and standard wash methods.

The workflow for xMAP protein biomarker samples is essentially the same for polystyrene (MicroPlex) or magnetic (MagPlex) microspheres. Typically, the workflows use conventional ELISA-based strategies of immobilization of analyte using a primary antibody to a support (e.g., microsphere), followed by the addition of a secondary-tagged antibody to a different epitope on the protein, and ending with a detection reagent, which will bind to the tag on the secondary antibody as shown in Figure 12.

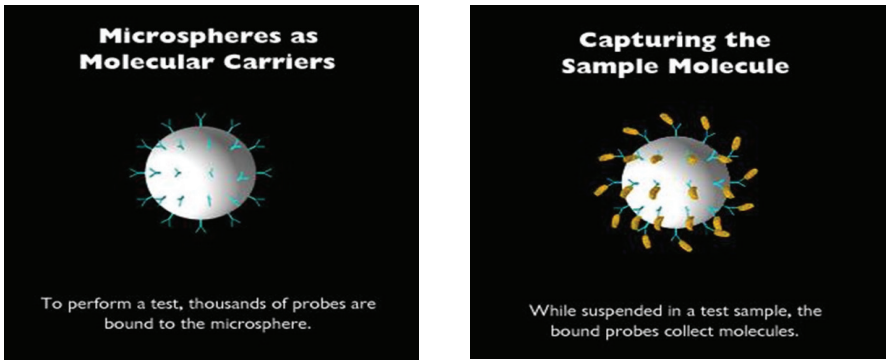


Figure 12. xMap workflow. Microspheres act as solid substrate. The beads are impregnated with one or more dyes at varying ratios for identification of analyte specificity. Sample is added with subsequent capture of target analyte. Unbound material is washed away by vacuum filtration or biomagnetic separation prior to analysis.

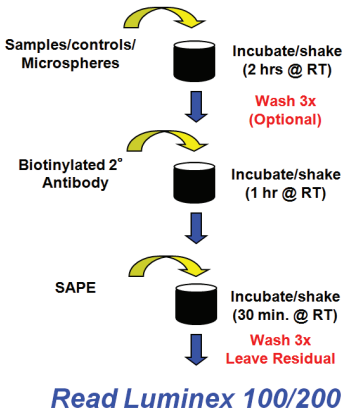


Figure 13. Typical workflow for protein biomarker quantification. Red text indicates use of Agilent BioTek washers to automate the process. SAPE refers to streptavidin-conjugated phycoerythrin as a detection reagent.

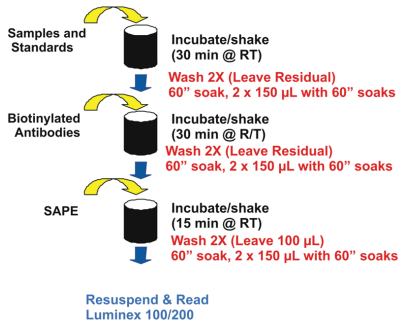


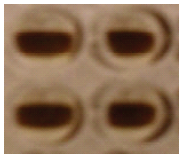
Figure 14. Avian influenza virus antibody assay test procedure.

Wash steps are required either after introduction of the sample if complex sample matrices are used (to reduce background signal), or after the final addition of detection reagent to simplify the workflow. The workflow is presented in Figure 13.

Factors affecting biomagnetic separation

Aspiration is the most critical step when washing magnetic beads. The location of the tubes relative to the beads will determine bead retention as the beads are only marginally held on the well surface. Different plate types and magnet arrangements require different software offsets to avoid bead loss (Table 4). The duration of aspiration is also a critical parameter. Aspiration of fluid forms a localized vortex in each well that scours away beads from the magnetic field. Therefore, rapid aspiration dive rates tend to work best.

Table 4. Comparison of available custom magnets for automated Luminex xMAP assay plate washing.

Magnetic Field Comparison (Gauss)		
	Flat magnet designs–exclusive for Agilent BioTek by Dexter Magnetic Technologies, Inc. (Elk Grove Village, IL)	Ring magnet designs–exclusive for Agilent BioTek by V&P Scientific, Inc. (San Diego, CA)
		
96-well Format	6,800	7,094
384-well Format	4,300	6,994

Magnetic bead: Avian antibody test

The Luminex avian antibody assay workflow is similar to the AIV antibody ELISA assay described previously. However, the Luminex assay differs in that it is a multiplex assay for the presence of IgY antibodies to AIV in chicken serum samples and detects the addition of the secondary antibody (Figure 14).

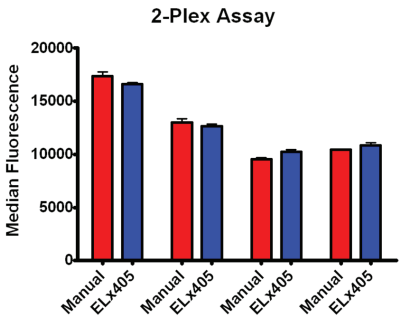


Figure 15. Comparison of magnetic bead assay performed using manual and automated wash methods.

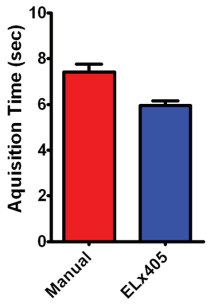


Figure 16. Comparison of acquisition time (120 beads counted) between automated and manual wash methods.

The assay was performed using both a handheld Dexter magnet and an Agilent BioTek 405 washer. Three separate washes of two cycles were performed using two different two-plex assays, either 8 or 16 wells, and 120-bead counts. The experimental data show comparable results between manual and automated methods
(Figure 15).

A comparison of acquisition time between methods can give an estimation of bead retention. When more beads are present, a resultant faster acquisition time is seen. Each assay was performed with 1,000 beads of each type with a total count of 120 beads (~60 beads of each type). The data presented in Figure 16 represents the average of 24 individual wells tested, suggesting that wells processed using automated wash methods on the 405 washer retain more beads than when the assay is performed manually.

Factors affecting filtration separation

The filtration time and vacuum levels should be optimized so that wells are completely aspirated but not subjected to sustained vacuum in a dry state. Prolonged aspiration can cause polysterene beads to become embedded in the filter matrix. Once embedded, they cannot be resuspended, so the read time will be prolonged. Typical automated washer filtration steps require 3 to 5 seconds with medium vacuum settings for complete well evacuation. Variations will result from differences in sample volume and sample matrix. Dispense height may also need to be optimized. However, the default washer settings for all Agilent BioTek washers work well for most assays. The use of very low dispense heights can result in fluid and bead loss from top aspiration.

Polystyrene bead: Thyroid stimulation hormone (TSH) assay vacuum wash process

Thyroid stimulating hormone (TSH) is a glycoprotein consisting of two subunits. This protein, which is secreted by the pituitary gland, stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3). TSH levels are tested in patients suspected of suffering from both hyperthyroidism and hypothyroidism. The ELx50 vacuum microplate washer can perform the necessary vacuum aspirations and fluid dispenses required for a typical xMAP MicroPlex assay using the parameters shown in Figure 17.

Figure 18 demonstrates a comparison between manual and vacuum wash methods, showing similar median signal responses between replicate samples. However, less variability is seen when performing vacuum wash methods.

Similarly, when a comparison is performed using a range of TSH concentrations, the responses correlate well to concentration, with the vacuum wash methods showing less variability (Figure 19).

LINK program SER1	High Vacuum Plug
Action	Parameter Value
Dispense	200 µl
Shake	30 sec.
Aspirate	120 sec.
Rotate plate	
Aspirate	120 sec.

LINK program SER2	Medium Vacuum Plug
Action	Parameter Value
Dispense	100 µl
Shake	5 sec.
Aspirate	30 sec.
Dispense	100 µl
Shake	5 sec.
Aspirate	30 sec.
Dispense	100 µl
Soak	5 sec.
Shake	30 sec.

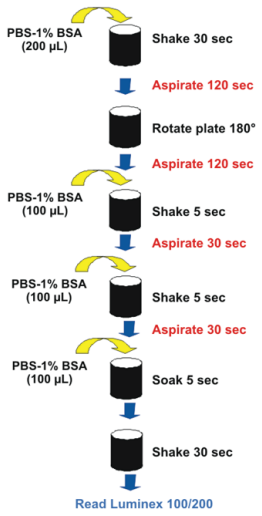


Figure 17. Typical Agilent BioTek ELx50 washer parameters and workflow schematic for performing vacuum-based assays with MicroPlex polystyrene beads.

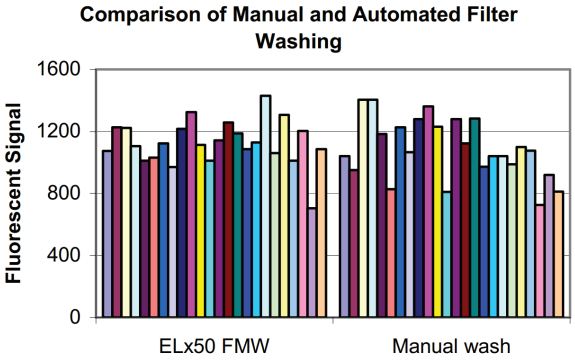
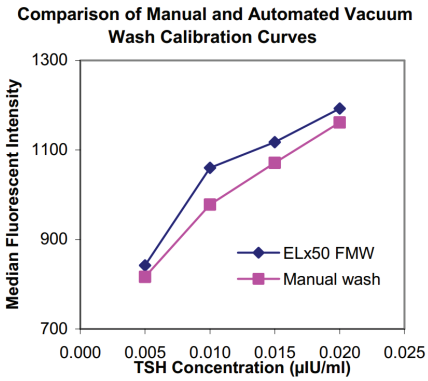


Figure 18. Comparison of magnetic bead assay performed using manual and automated wash methods.



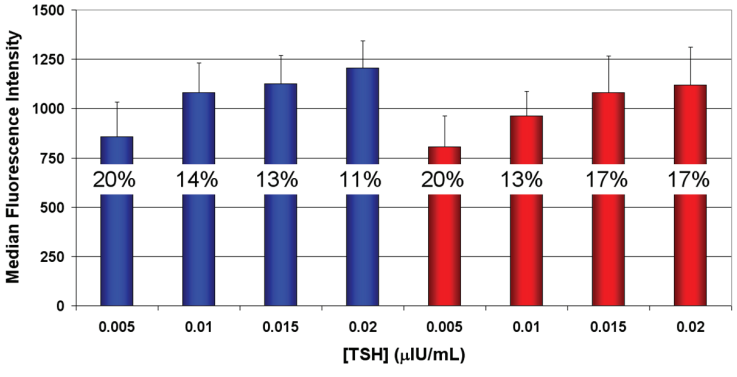


Figure 19. Comparison of manual and automated vacuum wash method. (A) The response correlating to changes in concentration are compared for each method. (B) Variability for the manual (red) and automated (blue) method are shown as %CV.

Cell-based assays

A central focus for improving drug efficacy in clinical trials over the last decade has been to increase the biological relevance of assays performed early in the drug discovery process. Biochemical assays used in screening campaigns are being replaced by cell-based, functional assays at ever increasing rates. These assays use a variety of cell models, including the overexpression of drug targets in immortalized cell lines, as well as human primary cells with endogenous expression of drug targets. It is imperative that correct instrumentation, as well as optimization of the instrument, be incorporated for dispensing and cell washing to further ensure that proper conclusions can be made with these promising cell models.

Fluid path selection

Most microplate washer manifolds and wash parameters were initially developed to work with ELISA assay workflows. The binding strength of 1° antibodies to antigens, and 2° antibodies to 1° antibodies allowed for the incorporation of more vigorous dispense speeds. With the adoption of these same instruments into cell-based assay workflows, there is increasing evidence that speeds which are optimal for ELISAs are too harsh for cell work, many times blowing cells off the bottom of the well (Figure 20).

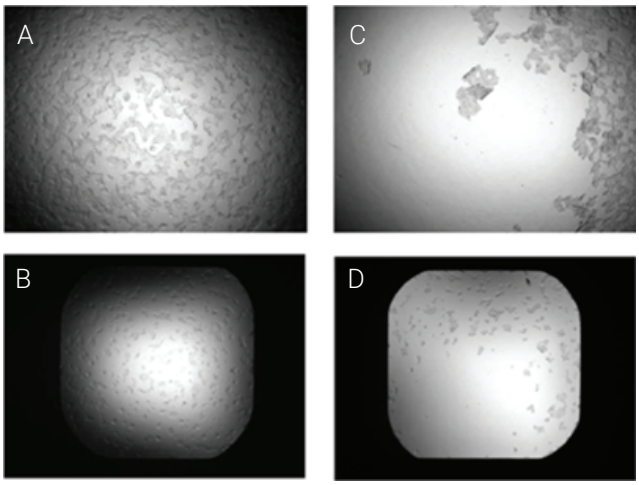


Figure 20. Effect of standard wash manifold dispense rates on cell monolayers. Cell monolayers before dispensing in (A) 96- and (B) 384-well format, and after dispensing in (C) 96-, and (D) 384-well format.

The ability to choose a flow rate that reduces fluid dispense speeds decreases the risk of harming cell layers, and improves the robustness of the automated workflow. “Low flow” rates can be selected with the 405, EL406, and MultiFlo FX that meet this need. A flow control valve closes the standard fluid path, forcing fluid through smaller diameter tubing (Figure 21).

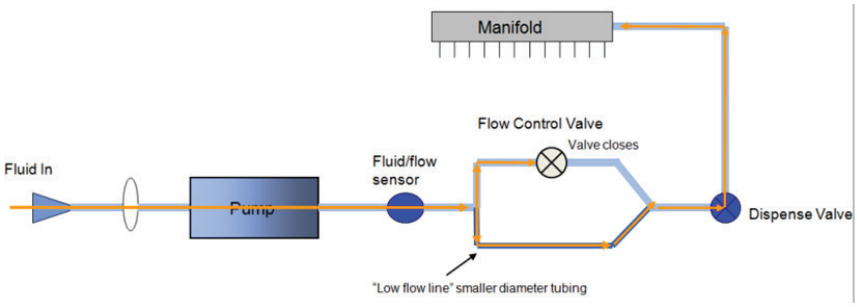


Figure 21. Diagram of “Low Flow” rate fluid path.

The final effect is that cell layers remain intact using a slower, more gentle fluid dispense rate (Figure 22).

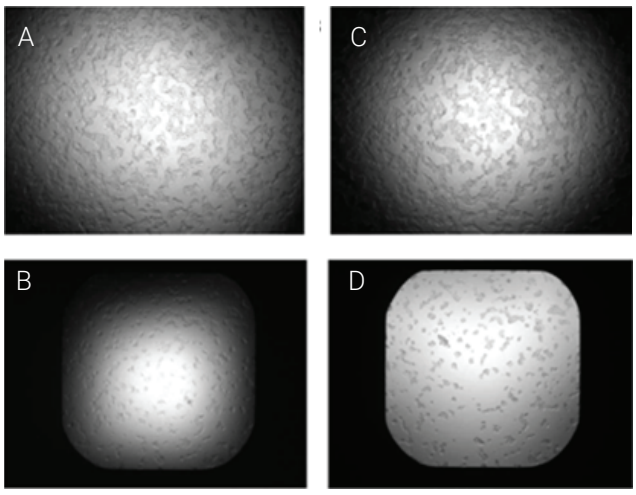


Figure 22. Use of low flow wash manifold dispense rates with cell monolayers. Cell monolayers before dispensing in (A) 96- and (B) 384-well format, and after dispensing in (C) 96- and (D) 384-well format.

Optimization of cell wash aspirate and dispense parameters

In addition to flow rate choice, other parameters can also prove critical when performing automated cell-based assay procedures, the first being proper placement of pins or tips within the well during dispensing. While Agilent BioTek washers and washer dispensers contain angled pins and tips to aid in the prevention of direct liquid dispensing onto cell layers, additional offsets in the X-axis may be necessary to further ensure that cells are not disturbed, by having liquid dispensed down the side of each well. This is illustrated in the parameters used for EL406 wash manifold dispensing (Figure 4B), as well as MultiFlo FX peristaltic pump dispensing (Table 1), referenced below.

Optimization of aspirate tube height for removal steps of medium exchanges or buffer washes is also essential. Allowing the tubes to be positioned too close to the cell layer when aspirating can lead to either partial or complete removal of cells in that area of the well. However the opposite—positioning the tubes exceedingly far from the cells—can also lead to deleterious effects. Incomplete replacement of spent medium can lead to a negative effect on cell health, while leaving behind an excessive volume of medium or buffer containing a fluorescent stain can create high background signal during reading or imaging steps that can impede proper data generation. The fine motor adjustment available in the Z-axis on all Agilent BioTek washers and washer dispensers allows for proper optimization of tube placement, leading to a robust automated procedure.

Automated fixation and staining of cells in microplates

The advantages of performing fluorescence microscopy in a microplate-based format include the ability to rapidly analyze multiple experimental conditions, obtain statistical information from repetitive experiments, and increase sample throughput. The latter attribute is fully enabled by automation of the experiment workflow. This is certainly true for immunocytochemistry applications, where cells must be fixed, permeabilized, and stained, which can be laborious, even when using a single slide. By using combination washer dispensers that are specifically designed for microplate operation, these workflows can be efficiently automated. For the application shown here, the EL406 was able to automate reagent dispensing onto cells in microplates in addition to washing cells to remove excess reagent. A typical automated workflow is demonstrated in Figure 23, while Table 5 illustrates the parameters used for each step. Figure 24 demonstrates the quality of the automated staining process using three separate fluorescent probes.

The automated procedure saves time, creates a more repeatable process, and eliminates the tedious work of performing multiple dispense and wash iterations by hand.

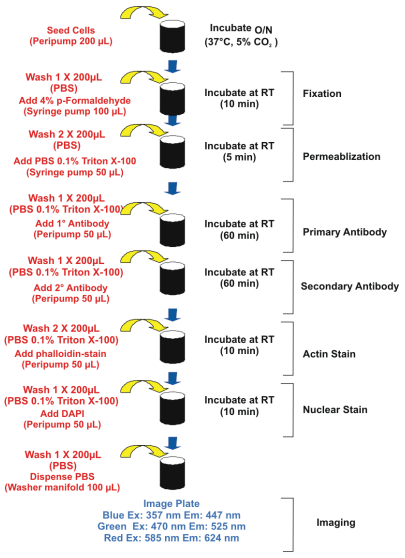


Figure 23. Automated workflow for cell seeding, fixation, permeabilization, and three-color staining. Agilent BioTek EL406 combination washer dispenser process for cell fixation, permeabilization, and staining with DAPI nuclear stain, Alexa-Fluor 488 phalloidin actin stain and Texas Red–labeled secondary antibody.

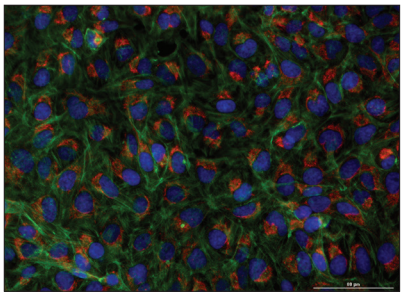


Figure 24. Three-color staining of U-2 OS cells in a 96-well plate.

Table 5. Agilent BioTek EL406 combination washer dispenser parameters.

Process	96 Well	384 Well
Number of Cycles	1–2	1–2
Soak/Shake	No	No
Soak Duration		
Dispense		
Volume	200	100
Flow Rate	1 CW	1 CW
Dispense Height	121	121
Horizontal X-Position	–15	0
Horizontal Y-Position	0	0
Delay Vacuum On	200	100
Aspiration		
Type	Top	Top
Height	45	45
Horizontal X-Position	–50	0
Horizontal Y-Position	0	0
Rate	6 CW	6 CW
Aspiration Delay	0	0
Final Aspiration	Yes	Yes
Height	40	40
Horizontal X-Position	–50	0
Horizontal Y-Position	0	0
Rate	6 CW	6 CW
Delay	0	0

Automated performance of a 3D-hydrogel-based cell signaling assay

Three-dimensional (3D) cell culture is poised to meet the need for a more in vivo–like cellular model with which to test large and small molecules. This is accomplished by providing a method that allows for the reorganization of cells into a format which re-establishes the necessary cellular architecture and communication networks seen in normal tissue. One methodology put forth to achieve this goal incorporates a simplified procedure for the creation of a cell and collagen hydrogel mix.

Once suspended within the hydrogel, the cells are free to aggregate into 3D tumoroid structures. The dispensing of viscous cell/collagen suspensions can be difficult to perform in a repeatable fashion using manual pipetting. Medium exchanges, in addition to other aspiration steps, can also be slow in order to ensure the hydrogel is not disturbed or removed by the pipette tip. Therefore, the inclusion of appropriate liquid handling instrumentation (Figure 25 and Table 6) can create a more robust process and eliminate potential human error (Figure 26).

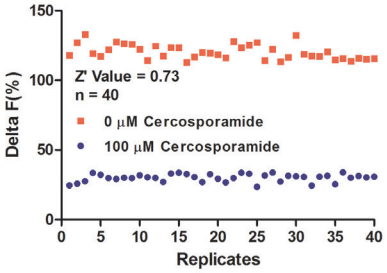


Figure 26. Z-Factor validation of the automated HTRF eIF4e assay.

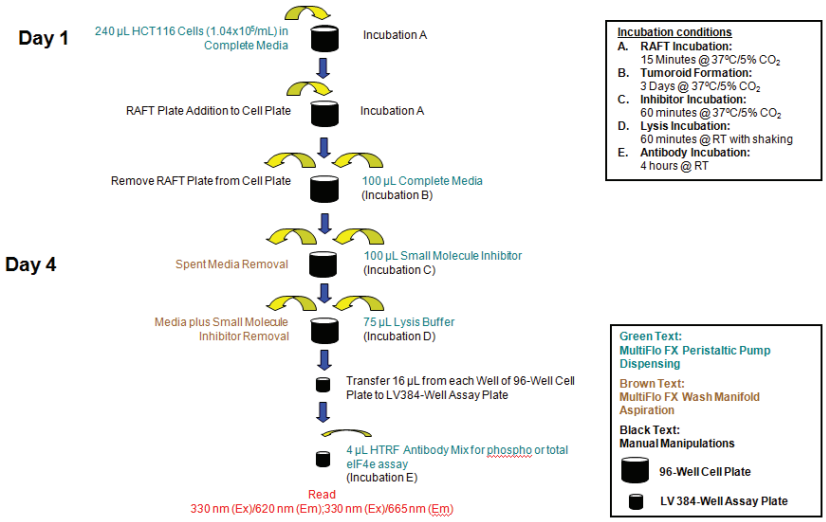


Figure 25. Automated HTRF eIF4e assay workflow using RAFT 3D cell culture system.

Table 6. Agilent BioTek MultiFlo FX multimode dispenser and washer settings.

Automated Procedure Peristaltic Pump Dispense Settings						
	Volume	Flow Rate	Cassette	Z-Position	X-Position	Y-Position
Cell Dispense	240 µL	Low	5 µL	336	−22	0
Medium Dispense	100 µL	Low	5 µL	336	27	0
Compound Dispense	100 µL	Low	5 µL	336	27	0
Lysis Buffer Dispense	75 µL	Low	5 µL	336	27	0
Ab Mix Dispense	4 µL	Low	1 µL	333	0	0

Automated Procedure Wash Manifold Aspirate Settings							
Travel Rate	Delay	1° Aspirate Z-Position	1° Aspirate X-Position	1° Aspirate Y-Position	2° Aspirate Z-Position	2° Aspirate X-Position	2° Aspirate Y-Position
6 CW	0	108	50	0	108	0	0

Importance of Microplate Washer Maintenance

Poor microplate washer maintenance can lead to assay failure, system downtime, and increased costs. Unexpected clogging of the washer manifold tubes is the single largest contributor to failures, thus routine maintenance is highly recommended. Best practices include keeping the manifold tubes wetted, using the day rinse feature, and periodic decontamination. Of course, busy laboratories have little time for these routine preventative maintenance practices, so Agilent BioTek microplate washers can be programmed to perform these tasks on a routine basis.

Agilent BioTek microplate washers can also be configured with a built-in ultrasonic cleaner to aid in routine washer maintenance. The proprietary Ultrasonic Advantage technology makes use of the cleaning principles provided by a standard benchtop ultrasonic cleaner and with routine use, essentially eliminate washer-associated failure, guaranteeing continued washer performance.

Ultrasonic cleaning involves cavitation, the rapid formation and violent collapse of minute vacuum bubbles that implode on surface contact. This agitation by countless small and intense imploding bubbles creates a highly effective scrubbing of both the inside and outside of the washer manifold tubes. Cavitation is produced by the Ultrasonic Advantage technology, which automatically pulses the ultrasonic energy into a liquid-filled built-in cleaning reservoir in which the washer's manifold tubes are fully submerged. Even heavy buildup from protein and salt crystals is dissolved from within the tubes. Ultrasonic cleaning can be user specified for both time of cleaning and duration for unattended operation. Common cleaning fluids are deionized water with the addition of a detergent such as Tween 20, or a commercial product such as Terg-A-Zyme, which contains a protease to help clean protein deposition. Figure 27 demonstrates the utility of the proprietary Ultrasonic Advantage for cleaning protein deposition. An initial 10 runs of Verify demonstrate good performance with about a 4% CV average. The washer is then allowed to sit idle for about three hours, filled with the milk protein casein. Verify is then run again for four runs, each failing with a very high % CV, indicating blockage of aspirate and dispense tubes. The 405 is cleaned using the autoclean / Ultrasonic Advantage maintenance procedure (including Terg- A-Zyme) for one hour. After the cleaning, Verify confirms that the average % CV is again 4% or lower over the next several runs, indicative of the return to good washer performance.

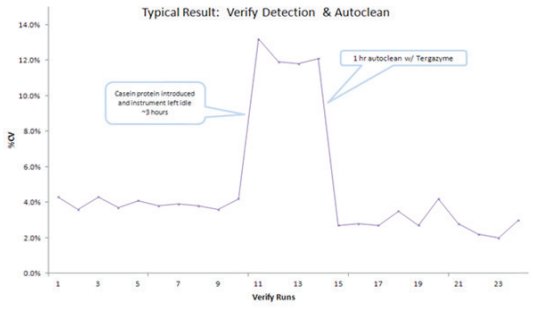
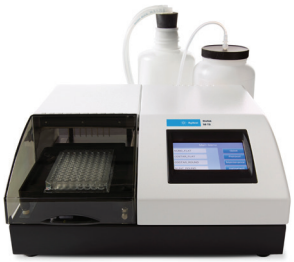


Figure 27. Washer performance precision as determined by Agilent BioTek Verify technology. Introduction of casein after nine Verify runs demonstrates poor precision over four Verify runs, before a return to good performance after a Terg-A-Zyme-based ultrasonic cleaning.

Agilent BioTek Microplate Washer Product Line



ELx50 microplate strip washer

The ELx50 Strip Washer is a self contained, programmable instrument that provides precise washing capabilities with single microplate strips, columns, or full plates. The flexible platform provides the ability to perform vacuum filtration, as well as biomagnetic separation, making it an excellent choice for automating the wash steps of 96-well magnetic or polystyrene bead assays, such as those developed on the Luminex xMAP platform. Comprehensive onboard software makes programming quick and easy. A fast and efficient vacuum filtration module allows vacuum to be adjusted with a range of settings for flexibility with polystyrene beads when using various filter pore sizes and sample viscosities. The vacuum filtration module is also well suited for filtration-to-waste processes such as PCR cleanup after DNA amplification to remove unwanted residues or reaction by-products with filtrate.

The ELx50 can also be equipped with a proprietary Dual-Action 16-channel manifold. This design allows for independent control of the dispense and spiration manifolds for overfill washing and overflow protection in both 96- and 384-well formats.

405 Touch microplate washer

The 405 Touch washer offers high-speed, full-plate washing of 96- and 384-well microplates. The instrument also features the proprietary Verify technology, which runs an automated QC check for manifold tube blockage, and visually reports any failed wells. The proprietary Ultrasonic Advantage can then be used to automatically and thoroughly clean the aspirate and dispense manifolds. The 405 Touch, with the unique Verify and Ultrasonic Advantage features, overcomes the common problem of undetected washer manifold clogging, which can lead to assay failure and makes it the first self-checking, self-maintaining microplate washer available.

The washer incorporates a high resolution touch screen user interface for intuitive and flexible programming. The extensive onboard software seamlessly guides users through protocol development, instrument maintenance and operation. For those who prefer computer control or require 21 CFR Part 11 compliance, the 405 Touch can be controlled with optional LHC software.

Cell-based assays and microsphere-based assays are easily run with the 405 Touch unique set of available features and modules for gentle washing vacuum filtration and biomagnetic separation. The 405 Touch is Luminex xMAP approved; a range of configurations is available for this and a broad variety of other microplate washing applications.

EL406 washer dispenser

The EL406 washer dispenser offers fast, full-plate washing along with three reagent dispensers in one instrument. The instrument contains the latest advancement in 1536-, 384- and 96-well microplate washing and dispensing, incorporating the proprietary Dual-Action manifold, optimized washing for loosely adherent cell monolayers, built-in proprietary Ultrasonic Advantage for unattended wash manifold maintenanc, and up to four wash buffers for complex wash routines. The available magnetic bead washing module offers high-strength biomagnetic separation in both 384- and 96-well microplates for full-plate washing of magnetic microspheres, which are used in a growing number of multiplex assays and bead-based ELISAs along with reagent dispensing. Developed in conjunction with leaders in genotyping, gene expression, and protein assays that were built upon the Luminex xMAP platform, Agilent BioTek magnets incorporate high-energy neodymium iron boron magnets for rapid separation of micrometer and nanometer beads with superior retention. An accessory magnet adapter kit allows any EL406 configuration to accommodate magnetic bead assay washing and dispensing. An available vacuum filtration module also makes the EL406 well suited for polystyrene bead assays and filtration-to-waste processes.

In addition, the EL406 provides the ability to incorporate peristaltic and syringe pump dispensing into ELISA, bead-based, and cell-based automated processes, without having to reconfigure the instrument or purge and reprime dispense lines. This eliminates manual intervention and allows for uninterrupted operation, even when performing long and complicated assay procedures. Similar to the 405 washer, the EL406 is 21 CFR Part 11 compliant when under the control of the Agilent BioTek LHC Secure software.

MultiFlo FX multimode dispenser

MultiFlo FX is a modular, automated multimode reagent dispenser for 6- to 1536-well plates, offering the unique Agilent BioTek Parallel Dispense technology. Up to four independent reagents can be dispensed in parallel without potential carryover. The choice of peristaltic or syringe pumps allows reagent conservation and unattended operation down to 500 nL with low maintenance and consumable costs. The instrument becomes a versatile washer dispenser with the addition of a Dual-Action manifold for 6- to 384-well plate washing. A fully configured MultiFlo FX replaces up to five liquid handlers, saving space, time, and instrumentation budgets.

Dispense operations are optimized for sensitive cell-based assays: angled dispense tips and flexible flow rates keep cells intact, and walk-away automation is available with the Agilent BioTek BioStack 4 microplate stacker for handling lidded or nonlidded plates. Extensive onboard software is accessed from the large color touch screen for fast, easy programming and operation, and integrated USB ports are available for convenient file transfer and storage.

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