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A Reliable Washing Method for Multiplex Bead Assay Platforms

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At a glance

- Multiplex bead assay platforms are replacing traditional assays for measuring the reaction of a target compound to the influence of outside parameters
- Washing steps in a multiplex assay depend on a vacuum manifold to remove liquid from the wells of the filter bottom microplate
- A vacuum manifold can cause several inconsistencies in a multiplex assay
- A filter microplate washer offers increased reproducibility from microplate-to-microplate

The popularity of multiplex bead assay platforms, such as the xMAP technology from Luminex Corporation, is quickly rising, replacing traditional assays such as ELISA and microarray technologies for measuring the reaction of a target compound or molecule to the influence of outside parameters. The motivation behind the shift to multiplexing is the ability to simultaneously analyze multiple targets within one reaction, rather than measuring one target per reaction.

Multiplex assay platforms involve microspheres in the range of 3 to 6 μm that are internally dyed with fluorescent materials and then coated with a substrate unique to the desired test or tests to be performed. These microspheres, alone or in combination with other coated microspheres, are then suspended in the sample, typically in the wells of a filter bottom microplate. When the coated substrate binds with a target molecule, two lasers—which are red and green when using xMAP technology, excite the microsphere's internal dyes that identify each microsphere particle, and also any reporter dye captured during the assay. The resulting fluorescence can then be detected via flow cytometry or appropriate imaging device.

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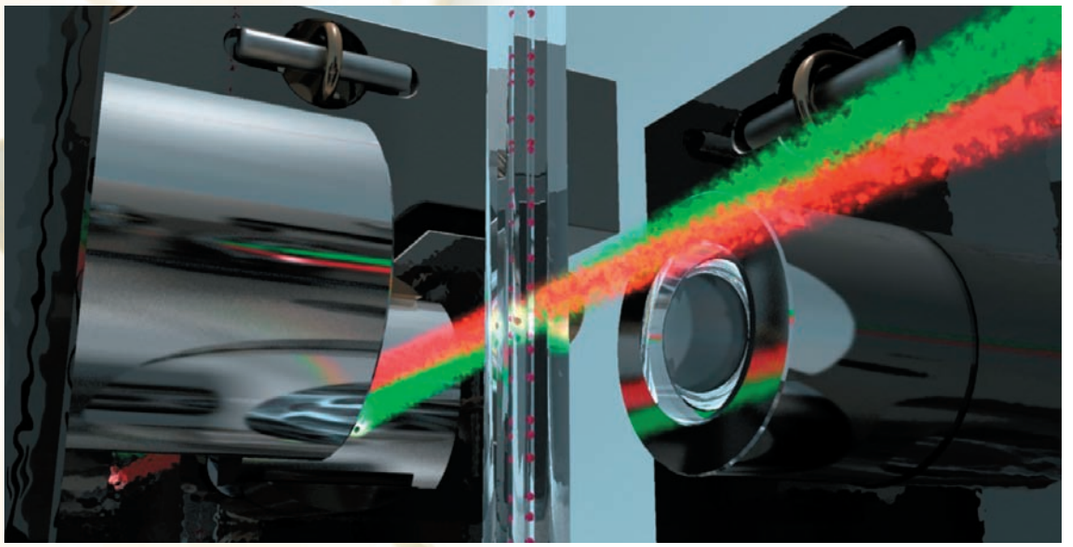

Figure 1. *Target molecules which fluoresce are detected via flow cytometry.* Image courtesy of Luminex Corporation.

The chief advantages of a multiplex assay platform are an increase in the amount, sensitivity and precision of information collected, as well as a decrease in costs associated with cell culture, enzymes/reagents, compounds, labels and other consumables. As these assays can incorporate up to several hundred targets per sample in one microplate well, the combined cost savings and convenience can be substantial.

However, no matter how sensitive and advantageous the multiplex assay platform is, inconsistent performance of the supporting equipment may add further steps to the process or even distort the final results. One such example of supporting equipment in a multiplex assay that can exhibit this variable performance is a vacuum manifold.

As the samples depend on microspheres as a solid support instead of the walls and bottom of a microplate well, it is impossible to wash and remove liquid via normal aspiration methods. To bypass this issue, a vacuum manifold has been the unrivaled choice to extract liquid from filter-bottom microplates since their debut over 20 years ago. This vacuum manifold technology has remained virtually unchanged over the years in spite of major advances in assay procedures.

Washing steps in a multiplex assay depend on a vacuum manifold to remove liquid from the wells of the filter bottom microplate so as not to disturb or remove the multiplex beads in solution. The microplate is placed in the manifold, the lid is lowered to seal the chamber, and vacuum is applied via a pump or in-house source. Often, it is recommended that the user apply light pressure to the manifold lid to ensure a good seal. Once the liquid is removed and discarded, the filter microplate is removed from the vacuum manifold and additional solutions and buffers are added. This step is repeated at various points throughout the procedure.

A vacuum manifold can cause several inconsistencies in a multiplex assay. It is difficult to accurately regulate vacuum pressure from well-to-well and plate-to-plate as the vacuum source and manual pressure to the lid may fluctuate, leading to unwanted liquid residue in the microplate wells if the pressure is too low. Conversely, if the pressure is too high, the microspheres may become trapped within the filter membrane. Additionally, vacuum pressure is often constant if not equipped with a controller, or limited to narrow parameters, even though the viscosity of the solution may vary. This can also lead to clogging of the filter membrane and ensuing loss of sample/bead matrix. Bleed valves to quickly break the vacuum can create contaminating aerosols or splashing if residual liquid remains in the microplate well or in the filter in the bottom of the microplate. Finally, vibrations from an attached vacuum pump can contribute to blockages in the filter microplate membrane. All of these factors can lead to inconsistent, unexpected, or incorrect results that have no relation to the multiplex assay technology.

Adding to the matter is the issue of convenience. Vacuum manifolds address only half of the washing process; the filter microplate must withstand further handling for the addition of reagents and buffers, often via the additional step of manual pipetting.

A more consistent alternative to vacuum manifold technology in a multiplex assay system is a filter microplate washer. This type of equipment automates the washing process to provide equivalent or better washing performance without the technical difficulties and variations that are associated with vacuum manifolds. This newer technology offers increased vacuum control coupled with precise aspiration and dispensing of washing buffers and reagents as used in a conventional microplate washer. This combination provides convenient operation with consistent performance by consolidating the overall number of steps in the washing process.

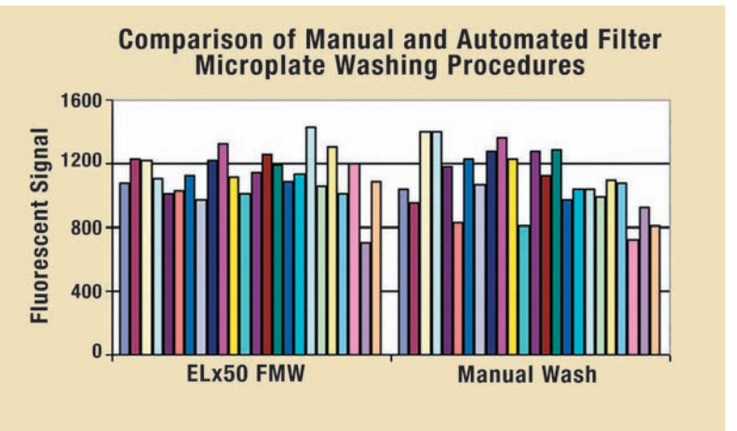

Figure 2. *Sample TSH multiplex microsphere assay using the BioTek ELx50 Filter Microplate Washer shows similar removal of unwanted microplate well constituents (background noise) when compared to a manual vacuum station but without inconsistencies and technical difficulties.*

ELx50 Filter Microplate Washer				
Concentration	4	5	7	8
STD	0.9	0.8	1.0	0.9
%CV	20.38	14.48	15.81	11.91
Manual Vacuum Station				
Concentration	5	7	8	9
STD	1.0	1.5	1.6	2.2
%CV	20.48	23.68	20.62	24.02

Table 1. *Improved microplate CVs are evident when comparing multiple microplates of TSH multiplex microsphere assay data using the ELx50 FMW compared to a manual vacuum station.*

As referenced in Figure 2, the ELx50 FMW can provide uniform and complete washing compared to traditional vacuum manifolds, making the transition into a more automated process simple and uncomplicated. Additionally, this filter microplate washer offers improved CVs (Table 1) compared to traditional vacuum manifolds, for increased reproducibility from microplate-to-microplate.

The ELx50 Filter Microplate Washer is designed to ensure consistency and convenience during washing and dispensing steps, especially in multiplex bead assay protocols. The filter microplate is placed in a specially designed microplate carrier that is equipped with a locking plate hold-down. This firmly seats the filter microplate onto the seal and prevents it from moving during operation while also providing a uniform seal, ensuring consistent vacuum across all wells both flexible and rigid microplates.

After the microplate is inserted and simple instructions are programmed, no further operator intervention is necessary. The carrier quickly aspirates from below the seated microplate per the selected interval, so that fluid is evenly and completely drawn through the microplate's filter membrane. A droplet grid beneath the filter microplate supports the membrane and controls droplet movement to prevent potential cross-contamination. When using solutions with different viscosities or assorted filter membranes, three selectable vacuum settings ensure complete evacuation of the liquid without obstructing or damaging the filter membrane or the multiplex beads.

Once all liquid is removed, additional wash buffer, or even a different buffer/liquid, is precisely dispensed into the microplate wells, and the process repeated as necessary. The multiplex beads are not harmed by the forces associated with vacuuming and dispensing, which ensures complete recovery prior to detection/imaging steps.

To summarize, using a filter microplate washer during the washing steps of a multiplex assay will simplify the process, as dispensing and aspiration of the wash buffers can be completely automated, allowing for unattended operation. In addition, with customizable vacuum control and non-aerosol generating dispensing and aspiration, results are consistent and reproducible, and the accuracy of the assay is increased. As a multiplex assay can provide enormous amounts of data from precious samples, dependable operation of the supporting equipment is imperative to prevent costly and timely errors while providing the best possible results. ■

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