Automated ELISAs—Save Time and Increase Throughput

Abstract

While enzyme-linked immunosorbent assays (ELISAs) provide valuable high-sensitivity detection, the numerous preparation steps separated by incubations and often coupled with high sample throughput, result in significant resources being monopolized. Here we demonstrate how the integration of the MultiWash™+ Microplate Washer and EMax® Plus Microplate Reader to a Biomek NXᴾ Workstation facilitates the automated processing and analysis of ELISAs. This automated solution can free up resources while providing more reliable ELISA results.

Introduction

Absorbance-based microplate assays have long been used for a variety of applications. One of the most common uses, and one that frequently involves high sample throughput, is enzyme-linked immunosorbent assays (ELISAs). ELISAs are frequently used to screen numerous samples to identify a colony of cells that expresses an optimized antibody against a given antigen. “Sandwich” ELISAs can also be used to detect the presence or production of a protein of interest. The workflow for a typical ELISA entails antibody incubations followed by repeated wash steps to remove unbound antibody, as well as incubation with a substrate for a colorimetric reaction. While relatively straightforward, the numerous liquid transfers and incubations make this process time consuming. By automating the entire process—from sample processing to analysis—one reduces user effort and user-to-user variability as well as the likelihood of errors; thereby increasing the reliability of the data generated.

AAG-696APP11.14-A

BECKMAN COULTER
Life Sciences

Fig. 1. Image of the deck of the Biomek NXP Workstation with the integrated EMax® Plus Microplate Reader (left side) and the MultiWash™+ Microplate Washer (rear, not used for this assay) from Molecular Devices, LLC. The Workstation's rotating gripper is used to place plates on the integrated instruments, removing the requirement for user intervention.

Automated Solution

The Biomek NXP Workstation provides reliable liquid handling for sample preparation in a wide variety of workflows. This reliability is achieved through customizable pipetting steps as well as through removing the variability that typically occurs in preparations from different scientists. The Biomek Workstations also provide flexibility that includes configurable deck layouts and the ability to integrate additional devices to increase the utility of the system. To accelerate the plate washing steps and automate the analysis of ELISAs we integrated the MultiWash™+ Microplate Washer and EMax® Plus Microplate Reader from Molecular Devices, LLC to the deck of the Biomek Workstation (Figure 1). Plate washing protocols and plate definitions are generated on the MultiWash™+ Microplate Washer and then selected and executed as part of the Biomek method (Figure 2). The EMax® Plus Microplate Reader is similarly controlled through a step in the Biomek method that runs the selected analysis protocol in the SoftMax® Pro (Figure 2). This complete integration of hardware and software components reduces the need for user interventions and this serves to increase data integrity.

Higher sample throughput can be achieved through additional integrations, such as storage devices to hold additional plates and tips. In addition, processes both upstream and downstream of ELISA analysis can be automated on this system. Cell culture can be automated through the integration of incubators and the Biomek Workstation can be encased in a HEPA-filtered enclosure to provide a sterile environment for cell manipulation. The resulting ELISA data can also be used by the Biomek Workstation to drive downstream operations, such as passaging and expanding cell cultures that generate antibody titers above a given threshold. Automation of these additional workflows can add further value to a laboratory as more resources are freed from these tasks.

Demonstration

To illustrate the use of this integrated system we automated the preparation and analysis of a Quantikine ELISA kit for Mouse/Rat IL-22 (R&D Systems), the workflow for which is shown in Figure 3. Briefly, we automated the serial dilution of the Mouse/Rat IL-22 standard from 1000 pg/mL to 15.6 pg/mL, as directed. Assay diluent and the diluted standards were added to the ELISA plate in triplicate wells and the plate was shaken for two hours on the deck of the Biomek Workstation. Following the incubation, the Biomek gripper transported the plate to the MultiWash™+

BECKMAN COULTER
Life Sciences

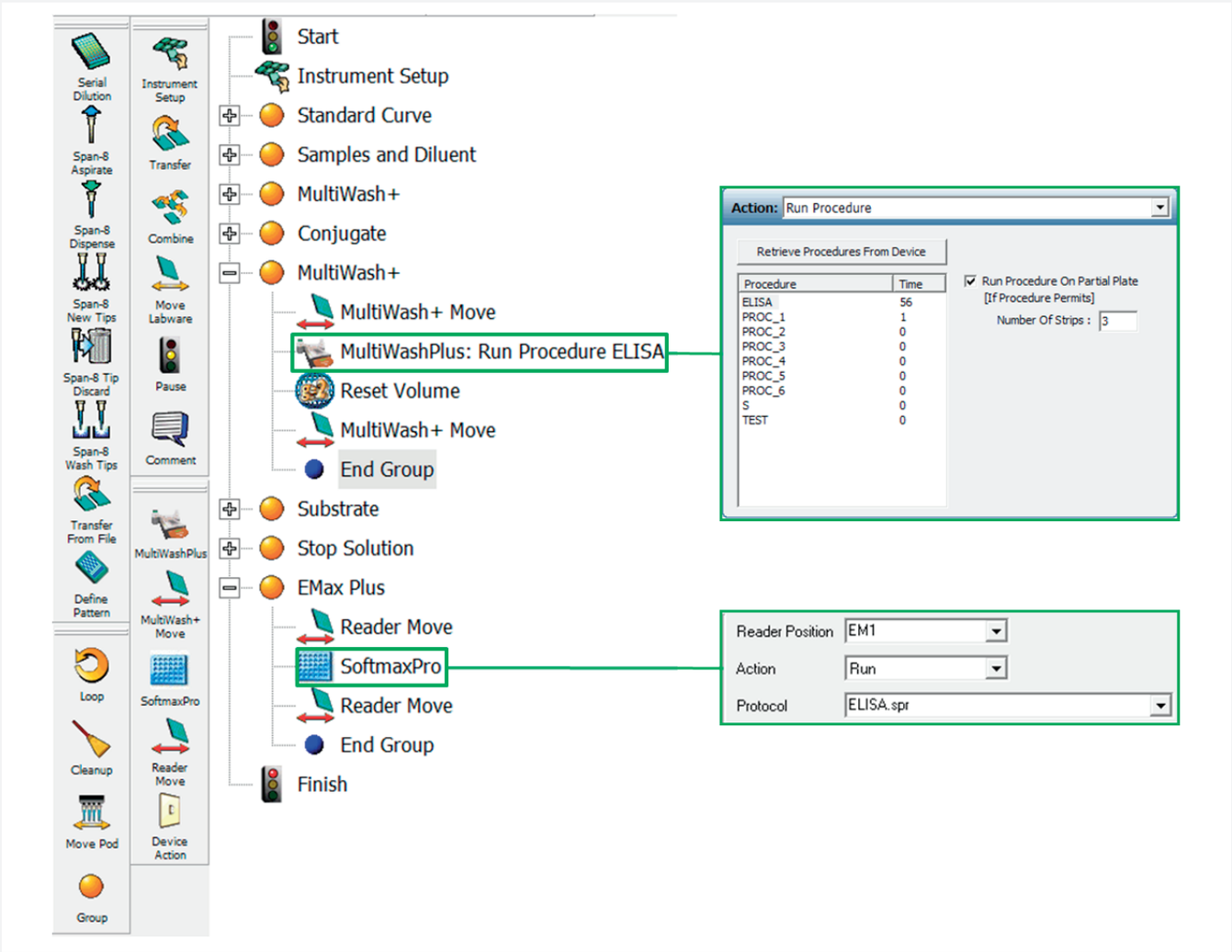

Fig. 2. Screen capture of the Biomek NX^{P} ELISA method (left) and the steps that control the integrated washer and reader (right). The "MultiWashPlus" step runs the procedure that has been selected from the list of available MultiWash™+ Microplate Washer procedures on the entered number of strips (i.e. plate columns). Similarly, the "SoftmaxPro" step automatically runs the selected SoftMax® Pro analysis protocol on the EMax® Plus Microplate Reader.

Microplate Washer for 4 wash cycles. Wash buffer was aspirated across the well bottom to ensure complete liquid removal. Conjugate was added by the Biomek Workstation, followed by another two hour shaking incubation and plate washing. Following the automated addition of freshly prepared substrate solution, the ELISA plate was incubated in the dark (offline) for 30 minutes. Stop solution was added to the plate and mixed by pipetting to avoid splashing the filled wells during shaking. Mixing was optimized to avoid generating bubbles in the wells, as these could affect absorbance readings. The Biomek gripper then transferred the ELISA plate to the EMax® Plus Microplate Reader for analysis. Well absorbance was measured at 450 nm as well as at 570 nm to normalize for optical imperfections of the plate.

BECKMAN COULTER
Life Sciences

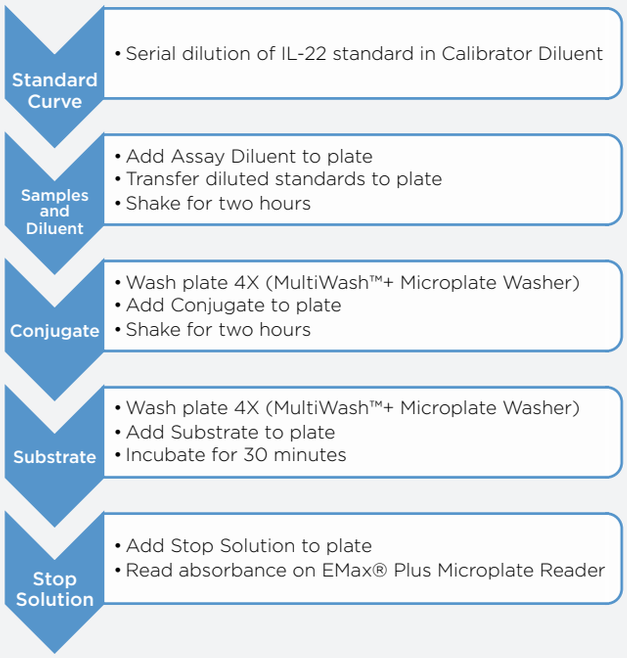

Fig. 3. Automated workflow for the Quantikine Mouse/Rat IL-22 ELISA.

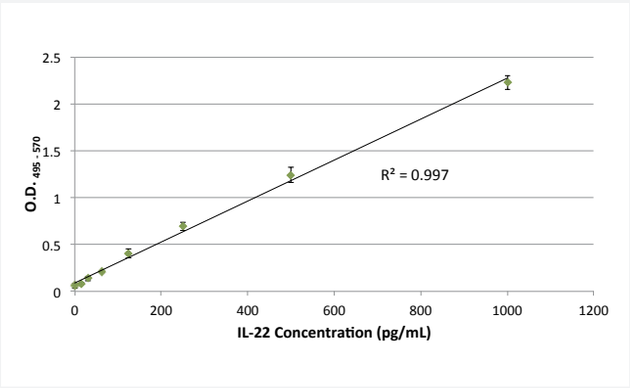

Fig. 4. Standard curve generated with the Quantikine Mouse/Rat IL-22 ELISA. Average absorbance for triplicate values of 0 to 1000 pg/mL Mouse/Rat IL-22 Standard. Absorbance at 495 nm was normalized by subtracting absorbance at 570 nm. Error bars represent standard deviation of the mean. The 0.997 R^2 value of the trend line indicates excellent linearity of the curve.

The standard curve results demonstrated high linearity (Figure 4, R^2 = 0.997), which matches or exceeds the manual samples prepared by Molecular Devices, LLC[1] (R^2 = 0.984). These data indicate this automated assay would provide reliable IL-22 quantification of unknown samples.

To estimate sample throughput, we simulated the addition of reagents and 32 samples in duplicate (64 total wells), in addition to the preparation of the triplicate standard curve samples. The estimated time of completion was under 5.5 hours despite the 4.5 hours of plate incubations. Throughput could be increased by taking the incubation steps offline or through integrating additional storage devices; however, the time saved in the processing of these samples is significant even at low sample numbers.

Conclusion

This work provides a demonstration of the utility of the Biomek NX^P Workstation to automate ELISAs through integration of a MultiWash™+ Microplate Washer and EMax® Plus Microplate Reader. Not only did the automated preparation achieve higher standard curve linearity than a manual preparation, the automation of the numerous liquid transfers dramatically reduces the effort required to prepare these assays.

References

[1]Molecular Devices, LLC. (2014). *EMax® Plus Microplate Reader Application Highlight.* PN: 1492A

Authors

Michael Kowalski, *Staff Applications Scientist*

Beckman Coulter Life Sciences
Indianapolis, IN USA

BECKMAN COULTER
Life Sciences

AAG-624APP11.14-A