Automate Like a Pro: Fully Integrated System for ELISA and Cell Viability Absorbance Assays

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Abstract

Absorbance-based microplate assays have long been used for a variety of applications. Common uses include protein quantification and identification assays such as Bradford and enzyme-linked immunosorbent assays (ELISAs). Absorbance assays can also be used to quantify cellular viability to measure cell growth or drug susceptibility. While these assays are relatively straightforward to execute, challenges arise when requiring higher throughputs, multiple time points, and/or consistent timing across multiple plates. Automating the sample preparation, incubation, and analysis of these assays, as well as upstream steps such as cell plating and treatment, can help overcome these challenges.

Here we demonstrate how the integration of a MultiWash+ Microplate Washer and EMax Plus Microplate Reader to a Biomek NX^P Workstation facilitates the automated processing and analysis of absorbance assays. Plates can be moved to the washer and reader using the Biomek NX^P gripper, thereby eliminating the need for user interventions. Not only did this fully automated solution achieve excellent standard curve linearity and replicate consistency across the three assays; the automation of the numerous liquid transfers greatly reduces the effort required to generate these results.

Materials and Methods

Reagents	Manufacturer	Cat #
HCT116 Cells	ATCC	CCL-247
Bio-Rad Protein Assay Kit II	Bio-Rad	500-0002
Mouse/Rat IL-22 Quantikine ELISA Kit	R&D Systems	M2200
XTT Cell Proliferation Assay Kit	ATCC	30-1011K

Instrumentation

A Biomek NX^P Workstation with Span-8 pipettors was used to automate the described workflows. **Figure 1** shows one possible deck layout for full automation of absorbance assays. The configurable deck layout includes an integrated EMax Plus Microplate Reader and MultiWash+ Microplate Washer, along with an orbital plate shaker.

The EMax Plus Microplate Reader is an absorbance reader with 8 filters to cover a wide range of applications. It utilizes SoftMax Pro Software, which can be controlled through the Biomek software as part of the automated run.

The MultiWash+ Microplate Washer is an automated washer that is configurable for both 96- and 384- well plates. Up to 20 different wash protocols can be created with variations in wash reagent, speeds and volumes.

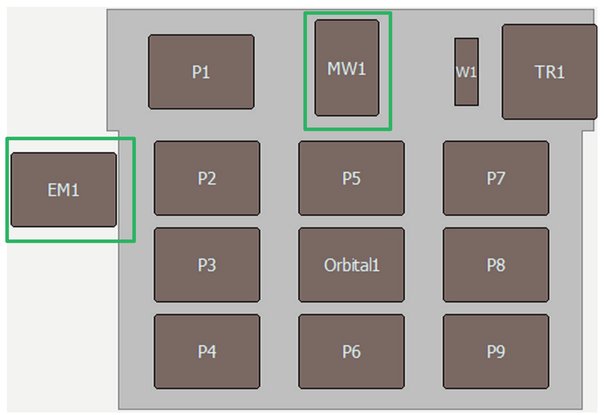


Figure 1. Biomek NX^P Deck Layout. Software representation of the deck of the Biomek NXP Workstation. Absorbance assay integrations include the EMax Plus Reader (EM1) and MultiWash+ Washer (MW1).

Automated Absorbance Assays

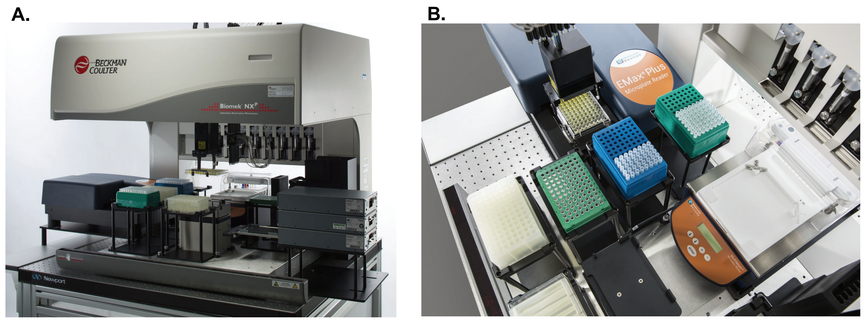


Figure 2. Integrated System for Automated Absorbance Assays. The Biomek NX^P with Span-8 pipettors (**A**) was integrated with an EMax Plus Microplate Reader and MultiWash+ Microplate Washer (**B**) for the complete automation of sample preparation and analysis of absorbance assays.

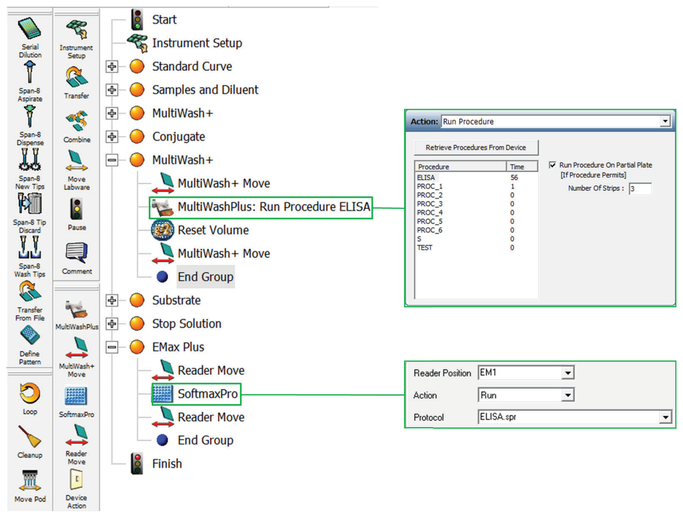


Figure 3. Biomek Software and Device Communication. Screen capture of the Biomek NX^P ELISA method and the steps that control the integrated washer and reader. The "MultiWashPlus" step runs the procedure that has been selected from the list of available MultiWash+ Washer procedures on the entered number of strips (plate columns). Similarly, the "SoftmaxPro" step automatically runs the selected SoftMax Pro Software analysis protocol on the EMax Plus Reader.

ELISA

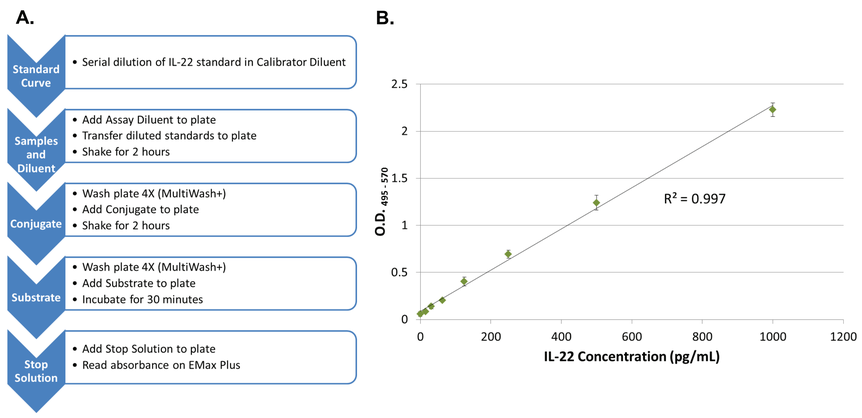
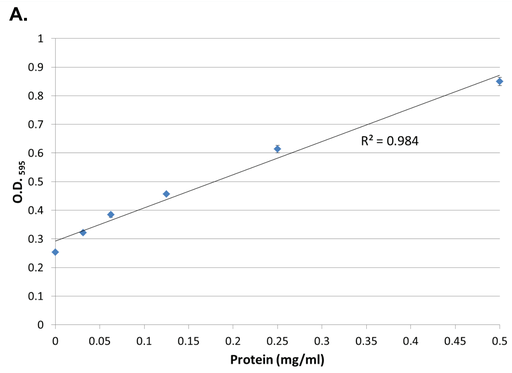


Figure 4. Automated ELISA. **A**) Workflow for automated ELISA. The MultiWash+ Microplate Washer was used to remove antibody solution and add and remove wash solution after incubations. **B**) The automated standard curve shows exceptional linearity (R^2 = 0.997), illustrating reliable standard dilution and reagent addition, as well as complete removal of additional antibody. These data indicate this automated assay would provide reliable IL-22 quantification of unknown samples.

Bradford Assay



Protein (mg/mL)	Average O.D.$_{595}$	CV (%)
0.5	0.850	1.7%
0.25	0.613	2.2%
0.125	0.456	0.2%
0.063	0.384	2.4%
0.031	0.321	2.0%
0	0.253	0.2%

Figure 5. Automated Bradford Assay. **A**) The automated standard curve shows excellent linearity (R^2 = 0.984), indicating reliable sample dilution and reagent addition. This is further reflected in the low variability seen across triplicate values within the standard curve, as indicated by coefficients of variation below 2.5% (**B**).

XTT Assay

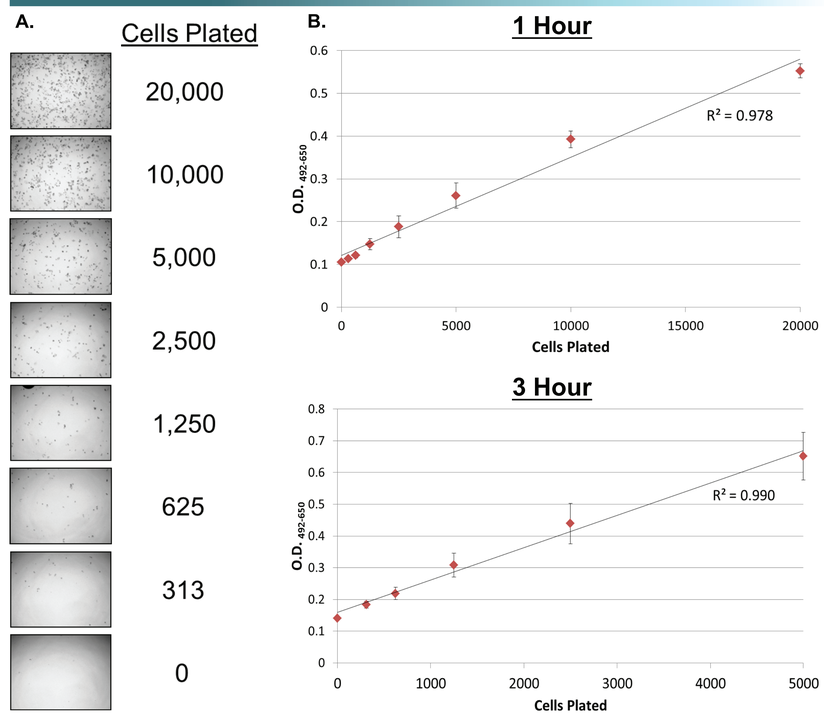


Figure 6. Automated Cell Plating and Cell Viability Assay. **A**) Brightfield images of cell cultures 24 hours after plating. Cells were serially diluted and plated using the Biomek NX^P Workstation. **B**) Cells were incubated with XTT reagent and assayed after 1 hour (upper) and 3 hours (lower) and absorbance reading from the EMax Reader were plotted against the plated cell number. The two time points could reliably quantify high cell counts (1 hr) and low cell counts (3 hr) to accommodate the wide range of viability conditions that could arise from a compound screen.

Summary

Complete automation of absorbance assays was accomplished through integration of an EMax Plus Reader to a Biomek NX^P Workstation. More complex ELISA workflows were automated through the additional integration of a MultiWash+ Washer. Low CVs and high standard curve linearity (R^2 > 0.97) highlight the consistency of the liquid handling steps, including upstream steps such as serial dilutions and cell plating.

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