



Automation of Beckman Coulter's Immunotech® IL-8 ELISA using Beckman Coulter's Biomek® 3000 Laboratory Automation Workstation

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Abstract

We have developed and optimized an automated method using the new Biomek 3000 Laboratory Automated Workstation to provide a complete, walk-away system for ELISAs. The system uses the unified Biomek Software to optimize liquid transfers while making sample processing as efficient as possible. The method allows processing of a single plate while minimizing reagent waste. Integrated plate washing is achieved using the Wash-8 Tool that provides consistent reagent addition and removal. The combination of the Biomek 3000 Laboratory Automated Workstation and integrated plate washing offers a complete system for processing ELISAs without user intervention.

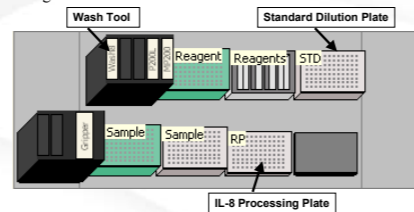
The information provided here will:

- describe the automated system used to process the ELISAs
- demonstrate the utility of the Biomek 3000
- describe the results when processing the Immunotech IL-8 ELISA kit on this system

Materials and Methods

Biomek 3000 Deck Setup

The deck setup in Figure 2 illustrates the labware positioners and labware required to process one ELISA plate. No user intervention is required after initial setup and framing. The method utilizes the Wash-8 Tool to allow plate washing on deck.



Materials and Methods

Running the Biomek 3000 Method

Prior to running the method, the ELISA deck was framed and the Wash Unit with Automatic Six-Port Valve was connected to the Biomek 3000. The system was purged with 2000 µl of wash solution to ensure no air was in the wash system lines. The method was executed by:

- (1) Opening the ELISA method in the Biomek Software.
- (2) Choosing "Run" in the "Execution" menu.
- (3) A confirmation window will appear to check the deck setup.
- (4) Choosing "OK" to start the method.

Once the method was completed, the plate was read at 450nm. Also, purging the Wash-8 Tool after method completion ensured that the system lines and tool would be clean of any wash solution.

Results

Whole Blood IL-8 Stimulation Results:

Supernatant fractions from the blood samples were processed in duplicate on the Biomek 3000. The method was run for both donors at 24 hour stimulation along with recombinant IL-8 standards from the kit. Also, unstimulated controls from both donors were run for comparison. Figure 5 shows the data for each dilution set. The standard curve data (Figure 6) demonstrated an excellent correlation coefficient of 0.99.

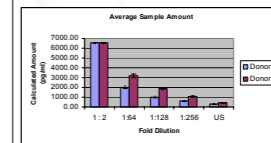


Figure 5. Average IL-8 sample concentration.

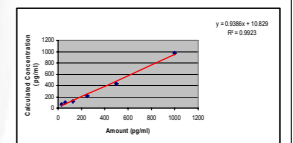


Figure 6. Standard curve.

Introduction

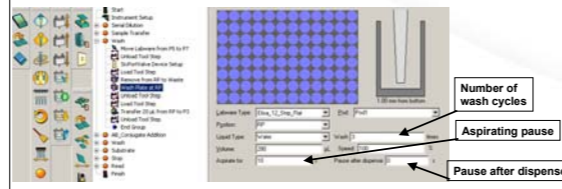
Processing ELISAs can be a very long and time-consuming task. Managing frequent time-sensitive steps and the large number of reagents can become very challenging when processing ELISA plates. We have developed and optimized an automated method to process a 96-well plate in under 5 hours without user intervention using the Biomek 3000 Laboratory Automated Workstation. The Biomek method allows plate washing by incorporating a Wash-8 Tool and an Automated Six-Port Valve unit. The combination of the Biomek 3000 and integrated plate washing offers a complete system for processing ELISAs without user intervention.

The results presented here demonstrate the quality and reproducibility of IL-8 data obtained using the Immunotech IL-8 ELISA on the Biomek 3000. IL-8 quantity was assessed after processing on the Biomek 3000 by spectrophotometric analysis on a non-integrated AD 340 Automated Labware Positioner (ALP). To test this system, IL-8 from mononuclear cells, that have been phytohemagglutinin induced at 24 hours from a primary culture of whole blood from 2 donors, was determined on the system. The data obtained from these tests will be summarized in the following slides.

Materials and Methods

Software used on the Biomek 3000:

The Biomek 3000 is controlled via Biomek Software, a common software architecture among Beckman Coulter's Laboratory Automation Workstations. Software features include definable wash steps, version control, and sample tracking. A view of the ELISA method, highlighting the wash step, is shown below (Figure 3). This can allow the user to control such things as number of wash cycles, pause while aspirating, and pause after dispense.



Results

Reproducibility/Carryover Test Results:

A major concern when running an assay is how consistent the results are across the entire plate. In order to test the reproducibility of this automated method, a test was run using samples of a constant concentration (1000pg/ml) across a single plate. Results (Table 1) show that the %C.V. obtained in this experiment was less than 10% indicating excellent reproducibility.

Plate Row	Standard Dilution	Standard Conc (pg/ml)	Plate Row	Average Sample (pg/ml)	StDev Sample (pg/ml)	%CV Sample
1	1000.00	1000.00	1	1040.25	49.32	4.74%
2	500.00	511.25	2	951.00	75.47	7.94%
3	250.00	272.50	3	948.50	61.65	6.50%
4	125.00	142.50	4	930.50	86.73	9.32%
5	62.50	96.25	5	952.75	62.22	6.53%
6	31.25	82.50	6	863.50	70.12	8.12%
7	0.00	-52.50	7	973.50	66.34	6.81%
8	0.00	-52.50	8	1002.00	63.80	6.37%
Plate Average				957.75	81.00	8.46%

Table 1. Average sample and standard concentrations.

Results

IL-8 Induction Data Summary:

The following summarizes the data obtained from the IL-8 induction experiment:

- Recombinant IL-8 standards for the run had a very consistent reproducibility across the plate with C.V.s <10%.
- The accuracy of the standard curve was excellent with a correlation coefficient of 0.99.
- Both donors had a high expression level of IL-8 after induction, but donor 1 had a slightly higher expression level (Table 2) at 24 hours compared to donor 2.

Donor	Average (pg/ml)	StDev	%CV	Donor 1	Average (pg/ml)	StDev	%CV
1:2	6570.33	0.00	0.00%	1:2	6570.33	0.00	0.00%
1:64	2924.29	168.55	7.93%	1:64	3184.50	168.65	5.30%
1:128	1025.54	103.83	10.12%	1:128	1883.04	85.56	4.54%
1:256	699.50	54.92	9.16%	1:256	1049.98	67.37	6.42%

Table 2. Average amount of IL-8 in 24 hour stimulated samples for both donors.

Materials and Methods

Biomek 3000 Configuration

A method was created using the Biomek 3000 Laboratory Automation Workstation (Figure 1A) for processing one IL-8 ELISA plate. On-deck plate washing was achieved using the Wash-8 Tool and the Wash Unit with an Automatic Six-Port Valve. The plates were read on an AD 340 ALP (Figure 1B).



(A)



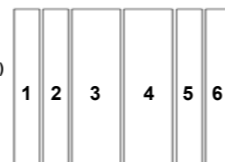
(B)

Materials and Methods

Reagent Plate Format

The reagent plate was formatted (Figure 4) to give optimum delivery of reagents to the processing plate. Volumes include 4 mls of Diluent and 1 vial of the remaining reagents from the ELISA kit.

- 1: Diluent (4 mls)
- 2: Biotinylated anti-IL-8 Antibody (6 mls)
- 3: Streptavidin-HRP (12 mls)
- 4: TMB Substrate (12 mls)
- 5: Stop Solution (6 mls)
- 6: Empty



Results

Whole Blood IL-8 Stimulation:

To test the functionality of the Biomek 3000 ELISA method with biological samples, a model system for inducing IL-8 production from whole blood samples was used. Two blood donors (1 and 2) were used and IL-8 was induced using the protocol described below. The 24 hour stimulated donor samples were then run on the Biomek 3000 using the automated method described above. Results are outlined in the next few slides.

Whole Blood IL-8 Protocol:

- Peripheral blood was obtained by venipuncture from healthy adult donors and was collected in a vacutainer containing EDTA (BD Bioscience).
- Mononuclear cells were isolated by density gradient separation on Ficoll-Hypaque gradients (Pharmacia) for 30 minutes.
- The mononuclear cells at the interface were collected, washed twice, and resuspended in RPMI-1640 (Invitrogen) culture medium supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen).
- The mononuclear cells were seeded at a density of 2×10^6 cells/mL in culture medium for IL-8 induction.
- Phytohemagglutinin (Invitrogen), at 1:100 dilution, was added to culture media to stimulate IL-8 induction. Unstimulated cultures were propagated as a control.
- Supernatant fractions from unstimulated samples and samples stimulated at 24 and 48 hours were collected and stored at -80°C for analysis.

Conclusion

The data presented here demonstrate that the Biomek 3000 Laboratory Automation Workstation can be utilized for executing an ELISA protocol as a means to detect immunological processes such as IL-8 induction. The method and system described here:

- Generated efficient and accurate data as demonstrated by the IL-8 standards in a 1-plate run using an integrated washer.
- Included optimized liquid handling and method performance as indicated by the high-precision data obtained when assaying recombinant IL-8 (1000pg/ml).
- Worked effectively in a model system using phytohemagglutinin-induced whole blood from 2 separate donors.

Acknowledgments

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