



Automation of Beckman Coulter's Immunotech® IL-8 ELISA using Beckman Coulter's Assay WorkStation Version 1.5

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Abstract

We have developed and optimized an automated method using the Assay WorkStation Version 1.5 to provide a complete, walk-away system that automates ELISAs. The system uses SAMI® WorkStation Scheduling Software to optimize the use of incubations to make processing as efficient as possible. The method allows processing of up to 9 plates in under 7 hours with minimal reagent waste. On-deck plate washing and reading are achieved by using an integrated MW96 washer and AD 340 Automated Labware Positioner (ALP), respectively. Consumables are stored in an automated storage module, Cytomat® Microplate Hotel HS with Plate Shuttle® System and brought onto the worksurface by the Biomek FX Cytomat ALP thereby allowing complete processing without user intervention.

The information provided here will:

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

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Materials and Methods

Assay WorkStation Software:
Assay WorkStation Software (Figure 2A) works by integrating a scheduling software (SAMI) with the Biomek FX Software. This is done by configuring nodes (Figure 2B) that use Biomek FX methods (Figure 2C), that are scheduled and run through the SAMI Software package. The SAMI ELISA method uses these nodes to process the 1-9 plate method.

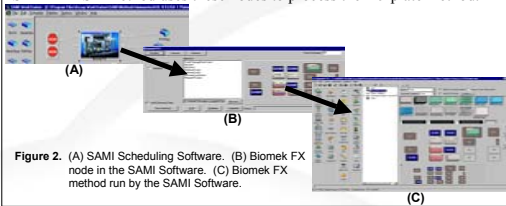


Figure 2. (A) SAMI Scheduling Software. (B) Biomek FX node in the SAMI Software. (C) Biomek FX method run by the SAMI Software.

Materials and Methods

Method Overview:

Figure 6 shows all Biomek FX methods (nodes) needed to run the SAMI method.

- (1) – Standard and sample transfer.
- (2) – 2 hour incubation.
- (3) – Wash with MW96.
- (4) – Biotinylated Antibody and Streptavidin transfer.
- (5) – 40min. Incubation.
- (6) – Wash with MW96.
- (7) – Substrate Addition.
- (8) – 30min. Incubation.
- (9) – Stop Solution addition.
- (10) – AD 340 detection at 450nm.

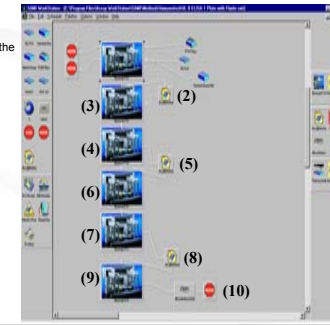


Figure 6. Method overview for the 9-plate ELISA method.

Results

Reproducibility/Carryover Test Results:

Equally important to recovery of the IL-8 sample across all 9 plates are the C.V.s within each plate. Table 3 shows the results of C.V.s on each plate during the 1000 pg/ml reproducibility test. Results demonstrate that intra-plate C.V.s are consistent with a range of 4-12% for wells with sample and 1-3% for wells without sample. Also, inter-plate C.V.s are consistent with an average of 8.05% further demonstrating the reproducibility of the assay.

Plate	Sample C.V.	No Sample C.V.
1	4.56%	2.23%
2	7.31%	2.11%
3	8.12%	2.19%
4	10.89%	2.11%
5	5.61%	2.81%
6	8.01%	1.98%
7	12.66%	3.11%
8	6.82%	2.83%
9	8.49%	3.12%
Average	6.69%	2.54%

Table 3. C.V.s for individual plates from the IL-8 reproducibility test.

Results

Whole Blood IL-8 Stimulation Results both donors:

The sample data are shown below for each donor's dilutions run in duplicate on 2 plates. Samples were arrayed in the following manner: Plate 1-2 = donor 1-24. Plate 3-4 = donor 1-48. Plate 5-6 = donor 2-24. Plate 7-8 = donor 2-48. The results shown are both the raw O.D.s (Figure 10) obtained and the calculated IL-8 concentration (Figure 11) for each donor sample.

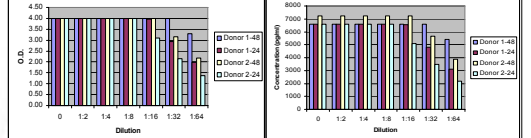


Figure 10. Average O.D. for donor samples. Figure 11. Average sample IL-8 concentration.

Introduction

Processing ELISAs can be a very long and time-consuming task. Managing frequent time-sensitive steps and large amounts of reagents can become challenging when processing more than one plate. Beckman Coulter, Inc. has developed and optimized an automated method to process 1-9, 96-well plates in under 7 hours without user intervention using the Assay WorkStation Version 1.5. The SAMI method allows plate washing and reading by incorporating integrated devices to control these time sensitive steps. Consumables are stored in a Cytomat Microplate Hotel to allow maximum plate processing.

The results presented here demonstrate the quality and reproducibility of IL-8 data obtained using the Immunotech IL-8 ELISA on the Assay WorkStation. IL-8 quantity was assessed by spectrophotometric analysis on an integrated AD 340 ALP. To test this system, IL-8 from mononuclear cells, that had been phytohemagglutinin induced at 24 and 48 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

Hardware Configuration - Biomek FX Deck:

The deck setup in Figure 3A illustrates the automated labware positioners and hardware required to run the SAMI method. SAMI access to the deck is defined in the Biomek FX Module Options (Figure 3B). No user intervention is required after initial setup. The method utilizes the integrated devices to allow plate washing and plate reading on deck.

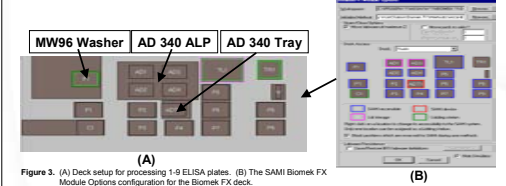


Figure 3. (A) Deck setup for processing 1-9 ELISA plates. (B) The SAMI Biomek FX Module Options configuration for the Biomek FX deck.

Materials and Methods

Biomek FX Methods Overview:

- 1) A Biomek FX method (Figure 7A) was run to generate a standards plate. The standards plate can also be set up manually.
- 2) An initialization method (Figure 7B) was run, identified in the Biomek FX Module Options screen, to allow tracking of tips during the SAMI method.
- 3) The SAMI method was run as indicated in Figure 8.

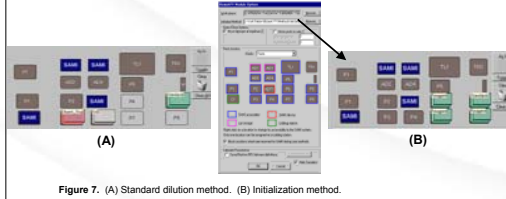


Figure 7. (A) Standard dilution method. (B) Initialization method.

Results

Whole Blood IL-8 Stimulation:

To test the functionality of the Assay WorkStation Version 1.5 method with biological samples, a model system for inducing IL-8 production from whole blood samples was used. Two donors (1 and 2) were used and IL-8 production was induced using the protocol described below. An equal number of donor samples was then run on 8 plates of the Assay WorkStation method. Results are outlined in the next few slides.

IL-8 Stimulation 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 from whole blood 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 and samples stimulated samples at 24 and 48 hours were collected and stored at -80°C for analysis.

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 on all 9 plates 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, but donor 1 had a slightly higher expression level (Table 5) at 24 and 48 hours for a 1:64 dilution compared to donor 2.

	Average donor 1	St. Dev.	%C.V.	Average donor 2	St. Dev.	%C.V.
24 hour Stim	3123.83	397.71	12.72%	2173.83	234.57	10.74%
48 hour Stim	5423.83	230.74	4.25%	3540.50	225.10	5.83%

Table 5. Average amount of IL-8 in 24 and 48 hour stimulated samples for both donors at a 1:64 dilution.

Materials and Methods

Assay WorkStation Configuration:

A method was generated using the Assay WorkStation Version 1.5 (Figure 1) and SAMI WorkStation Scheduling Software. On-deck plate washing and plate reading were achieved using the MW96 washer and AD 340 ALP, respectively. Consumables were stored in an automated storage module Cytomat Microplate Hotel HS with Plate Shuttle System and brought onto the worksurface by the Biomek FX Cytomat ALP.



Figure 1. Biomek FX Assay WorkStation Version 1.5

Materials and Methods

Hardware Configuration - Cytomat Configuration:

The Cytomat has to be configured to allow access to the proper labware (Figure 4). Each stack is assigned, using the Station Builder, to a piece of labware for SAMI to access and bring onto the deck for processing.

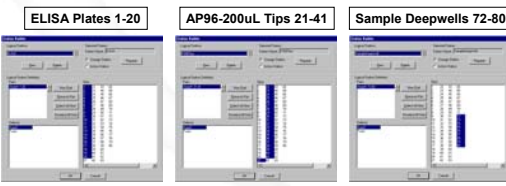


Figure 4. Station Builder to assign access to stacks in the Cytomat.

Materials and Methods

Running the SAMI Method:

Once all reagents are on deck and the standards method has been completed, integrated devices were turned on and the Span-8 system water was purged prior to setting up the standards. The MW96 washer was also purged with 50mls of wash prior to starting the method. The initial deck setup for tips and reagents for the SAMI ELISA method is shown below in Figure 8. The method was executed by:

- (1) Opening the 1-9 Plate IL-8 ELISA method in the SAMI Editor.
- (2) Choosing "Go" in the "Schedule" menu.
- (3) Selecting the number of plates to be processed.
- (4) Saving the schedule.
- (5) Choosing "Run Most Recent" in the "Schedule" menu.
- (6) Then choosing "Start."

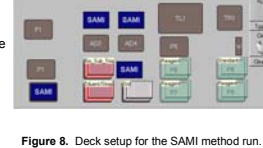


Figure 8. Deck setup for the SAMI method run.

Results

Whole Blood IL-8 Stimulation Standards Results:

The performance of the assay was tested by placing recombinant IL-8 standards in the first two columns of all 8 plates. The results (Table 4) demonstrate the calculated concentration (34-956pg/ml) for each input amount (31.2-1000pg/mL) of recombinant IL-8 standards was consistent with C.V.s <8% (Table 4) and a correlation coefficient of 0.99 (Figure 9).

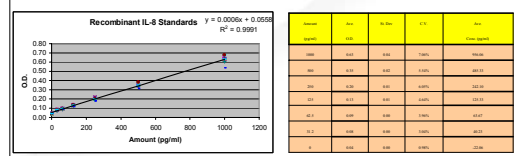


Figure 9. The Recombinant IL-8 Standards O.D for all 8 plates. Table 4. Recombinant IL-8 Standards.

Materials and Methods

Labware, Hardware, and Methods Overview:

Table 1 shows the labware, hardware, and software (methods) needed to run the 1-9 Plate IL-8 ELISA Assay WorkStation (SAMI) Method.

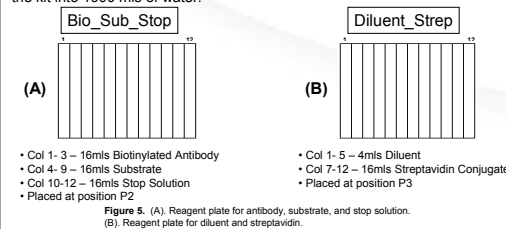
Labware	Hardware	Methods
13 Boxes AP96_200uL Tips	Hybrid Biomek FX	Initialization
10 CostarDeep96Square Deepwells	Cytomat	Standards Setup
9 ELISA Plates with Lids	Fluidic with Mounting Hardware	Sample Transfer
2 - 12 Column Reservoirs	AD340 Alp with Mounting Hardware	Fluidic Wash
	9 - TXI ALPs	Antibody Transfer
	Tip Loader	Substrate Transfer
	Cytomat Transportation ALP	Stop Solution Transfer
	Span-8 Tip Trash	Assay WorkStation IL-8
	Span-8 Tip Wash	

Table 1. Labware, Hardware, and Methods needed to run the Assay WorkStation method.

Materials and Methods

Hardware Configuration - Reagent Plate Format:

The reagent plate (Figure 5) was formatted to give optimum delivery of reagents for all 9 plates. Processing less than 9 plates will require reagent volume adjustments. The wash requires 2-50ml IL-8 wash solutions from the kit into 1900 mls of water.



- Col 1-3 – 16mls Biotinylated Antibody
- Col 4-9 – 16mls Substrate
- Col 10-12 – 16mls Stop Solution
- Placed at position P2
- Col 1-5 – 4mls Diluent
- Col 7-12 – 16mls Streptavidin Conjugate
- Placed at position P3

Figure 5. (A). Reagent plate for antibody, substrate, and stop solution. (B). Reagent plate for diluent and streptavidin.

Results

Reproducibility/Carryover Test Results:

Initial tests were performed to determine the reproducibility of data between individual plates and among all plates collectively, as this is an area of concern when running assays with multiple plates over extended times. To test this, 1000pg/ml of purified IL-8 were seeded into every other well on all 9 plates and the automated method was completed. The results (Table 2, Col. 4, 6, 8, 10, 12) show that average concentration on plate 1 (0.630.D=1131pg/ml) is consistent with average concentration on plate 9 (0.470.D=1032pg/ml). By placing sample in every other well, this experiment also demonstrates no carryover (Table 2, Col. 3, 5, 7, 9, 11) occurred during processing.

Plate	Col. 3	Col. 4	Col. 5	Col. 6	Col. 7	Col. 8	Col. 9	Col. 10	Col. 11	Col. 12	Average O.D.	Average Conc. (pg/ml)
1	0.00	0.63	0.00	0.63	0.00	0.63	0.00	0.63	0.00	0.63	0.63	1131.55
2	0.00	0.58	0.00	0.58	0.00	0.58	0.00	0.58	0.00	0.58	0.58	1081.30
3	0.00	0.58	0.00	0.57	0.00	0.59	0.00	0.59	0.00	0.57	0.58	1049.25
4	0.00	0.60	0.00	0.57	0.00	0.57	0.00	0.60	0.00	0.59	0.59	1061.85
5	0.00	0.59	0.00	0.54	0.00	0.57	0.00	0.59	0.00	0.59	0.58	1039.45
6	0.00	0.64	0.00	0.56	0.00	0.61	0.00	0.61	0.00	0.65	0.61	1226.04
7	0.00	0.57	0.00	0.56	0.00	0.56	0.00	0.53	0.00	0.54	0.54	965.94
8	0.00	0.51	0.00	0.59	0.00	0.48	0.00	0.51	0.00	0.53	0.51	997.60
9	0.00	0.46	0.00	0.45	0.00	0.48	0.00	0.48	0.00	0.47	0.47	1032.06

Conclusion

The data presented here demonstrate that the Assay WorkStation Version 1.5 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:

1. Generated efficient and accurate data as demonstrated by the IL-8 standards in a 9 plate run using an integrated washer and reader.
2. Included optimized liquid handling and method performance as indicated by the high precision data obtained when assaying recombinant IL-8 (1000pg/ml).
3. Worked effectively in a model system using phytohemagglutinin-induced cytokine production using primary cell cultures from whole blood from 2 separate donors.

Acknowledgments

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