
INSTRUCTIONS

Biomek 2000 - IL-8 (IM 2237)

**ADDENDUM TO THE INSTRUCTION BOOKLET TO RUN THE
AUTOMATE PROCEDURE FOR IL-8 ON A BIOMEK 2000
WORKSTATION.**

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1. INTENDED USE

The method is intended for the automation of the Immunotech-Beckman-Coulter (BCIOT) IL-8 enzyme immunoassay (EIA).

All instructions described in the manual IL-8 EIA are still applicable, the present instructions merely describe specific points of the automated protocol.

2. ROBOTIC CONFIGURATION REQUIREMENTS

The method for the automation of the IL-8 EIA requires the following items:

Description	Part Number	Quantity
Workstation	609000	1
Right side module	609047	1
Left side module	609048	1
Wash unit with automatic six-port valve	609056	1
Accessories		
Tool rack	609119	1
Labware holder	609120	8
Pipette tip rack holder	609121	3
Reservoir holder	372795	1
Tools		
Wash-8 eight-channel tool	609027	1
MP200 eight-tip tool	609025	1
P200L single-tip tool	609022	1

3. SPECIFIC BIOMEK 2000 LABWARE REQUIRED

Description	Part Number	Quantity
Quarter reservoir	372790	2
Quarter reservoir divided by length	372788	2
250µl tip rack	372654	3
Beckman square well titer plate	140504	1

4. IL-8 EIA KIT FOR BIOMEK 2000

The following components are included in the kit box.

Description	Quantity
Anti-IL-8 antibody coated plates	1 plate
IL-8 standard	1 vial, Lyoph.
Biotinylated monoclonal anti-IL-8 antibody	1 vial 6ml
TMB substrate	1 vial 12ml
Streptavidin-HRP	1 vial 12ml
Diluent 1	1 vial 25ml
Wash Solution (20X)	1 vial 50ml

5. OTHER MATERIAL REQUIRED, BUT NOT PROVIDED

Microtiter Plate Reader (450 nm)

6. LABWARE FORMAT FOR SAMPLES

Samples may be arranged in different types of labware such as culture plates, deep well or square well plates. In the provided BCIOT IL-8 method, the samples are arranged in 96-well flat bottom plates (Beckman-Coulter Part Number #373660). To change the format of the sample plates, select the suitable labware from the list in the “Edit labware” window. Copy this labware and rename as “BCIOT sample”.

7. SPECIFIC TOOLS

Several virtual tools have been created to guarantee precise and reliable liquid transfer. The user may check the presence of the following tools in the listing of tools.

Multi-channel tools:

MP200 IOT1

MP200 IOT2

MP200 IOT3

Single-channel tools:

P200L dil1

P200L dilC

P200L Std

8. DESCRIPTION OF THE METHOD

- Standards are prepared on the Biomek 2000 by serial dilution of a concentrated solution of IL-8, the concentration range is the same as that for the Manual IL-8 kit #2237. This preparation takes place in a separate plate.
- Standards are transferred in duplicate to the first two strips of each plate and samples to the following strips.
- Assay plates, sample plates and tip racks are processed according to the numbering indicated in the figure shown in section 10.2.
- Delays have been inserted at different steps in the process so as to control timing and to avoid plate-to-plate drift.
- At the end of the procedure, plates are ready for absorbance reading; data should be processed as usual.

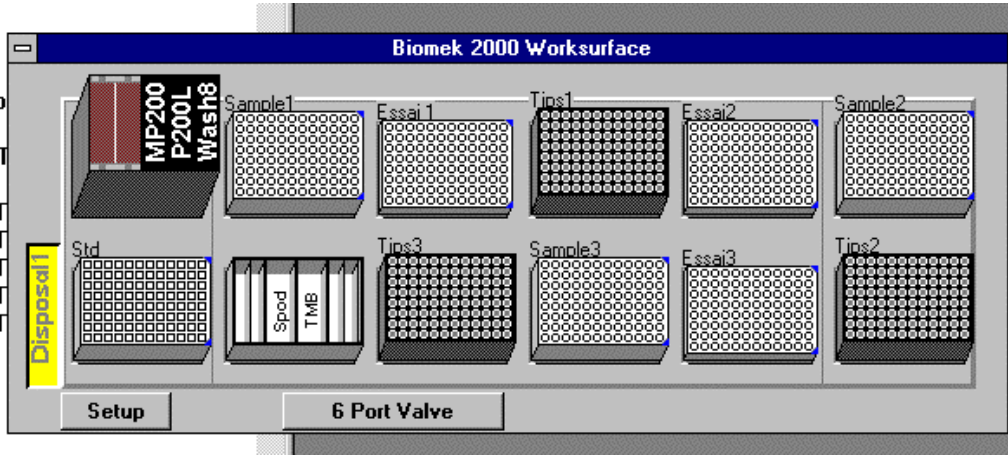
9. ASSAY PROCEDURE

9.1. Reagent preparation

- Dilute the wash solution with 950 ml of distilled water and pour into a bottle connected to Port 1 of the Wash unit.
- Fill bottle with distilled water and connect to Port 1 of the Wash unit.
- Reconstitute the IL-8 standard with distilled water and pour into the A2 well of the Square Well titer Plate (**Std** in figure 10.2).

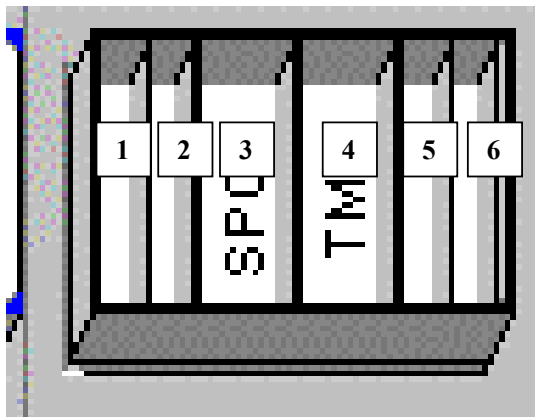
9.2. Preparation of the INITIAL CONFIGURATION

- Arrange holders, tools and plates without lids on the Worksurface according to the following scheme:



- Arrange the reservoirs in the reservoir holder according to the following scheme and pour the total contents of reagent vials as indicated:

Important: The prototype method is described for 3 plates, therefore, the components from 3 kits are required.



- 1: Diluent 1
- 2: Biotinylated anti-IL-8 antibody
- 3: Streptavidin-HRP
- 4: TMB substrate
- 5: Stop solution
- 6: To be left empty

9.3. Running the BCIOT IL-8 method

- Open the Run application of Bioworks, in the “Options” menu select:
 - “Verify Configuration”
 - “Reset all Tip Racks”
 - “Dry run”
- Click “Save Preferences” to store settings.
- Click the START button to start the operation.
- At the end of the run, remove the assay plates from the Worksurface and read on a microtiter plate reader set at 450 nm.

9.4. Schedule of the BCIOT IL-8 method:

Time	Step
00:00:00	Start of the Method: preparation of standards
00:03:54	Incubation of Plate 1 standards and samples
00:12:37	Incubation of Plate 2 standards and samples
00:21:17	Incubation of Plate 3 standards and samples
02:06:40	Wash Plate 1
02:14:08	Add biotinylated Ab to Plate 1
02:14:57	Add Streptavidin-HRP to Plate 1
02:16:20	Wash Plate 2
02:23:33	Add biotinylated Ab to Plate 2
02:24:25	Add Streptavidin-HRP to Plate 2
02:25:53	Wash Plate 3
02:33:06	Add biotinylated Ab to Plate 3
02:33:58	Add Streptavidin-HRP to Plate 3
02:56:20	Wash Plate 1
03:03:31	Add TMB substrate to Plate 1
03:05:03	Wash Plate 2
03:12:16	Add TMB substrate to Plate 2
03:13:54	Wash Plate 3
03:21:06	Add TMB substrate to Plate 3
03:34:48	Add Stop solution to Plate 1
03:44:01	Add Stop solution to Plate 2
03:52:55	Add Stop solution to Plate 3
03:55:32	Termination of Method