

Automation of 1-10 Plates of a Beckman-Coulter-Immunechem Cytokine ELISA for IL-8 on the Biomek 2000 Workstation - Application to the Beckman-Coulter-Immunechem ELISA for IL-8

V. Rocchi, Ph. Denizot and J. Fieschi
Immunoanalysis Department, Research & Development
Immunechem, a Beckman Coulter Company

Select the number of assay plates:

Simply change the loop number in Step Loop1.

Position of samples in sample plates:

In the default method, Samples are distributed from wells A3 to H12 of the sample plate to wells A3 to A12 of the assay plate. This source and destinations wells can be customized as needed and replicates can be added provided that the structure of Loop1 is not modified (e.g. 1 tip box to distribute from 1 sample plate to 1 assay plate).

Installation of plates in Stack D:

Sample and assay plates must be intercalated starting with a sample plate on shelf1 (see figure 2 in Application Details)
All the plates must have a lid.

Installation of tip racks:

Place 1 tip rack per assay plate in Stack C.
Place 1 tip rack on the holder at position A6

Distribution of reagents in the reservoirs:

Distribute the content of 1 vial per assay plate of each reagent in the appropriate reservoir, or fill to the top.

- Distribute the secondary antibody and the enzymatic conjugate into the reservoirs present on the worksurface at position A5.
- Distribute the TMB substrate in a full reservoir, pack with aluminum foil to protect from daylight and place this item at position A3.
- Distribute the Stop solution in a half reservoir placed at the center of a reservoir holder. Place this item on Shelf 1 of Stack A.

Connection of the wash unit:

Attach a bottle containing 0.5L of water to port 1 of the Wash unit
Attach a bottle containing 5L of diluted Wash solution to port 2 of the Wash unit

Preparation of standards:

- Dilute the concentrated standard included in the kit box according to the following protocol:

Standard concentration	IL-8	Diluent
------------------------	------	---------

(pg/mL)		
2000	200 µL of 20 ng/mL Standard	1800 µL
500	400 µL of 2000 pg/mL Standard	1200 µL
125	400 µL of 500 pg/mL Standard	1200 µL
31,2	400 µL of 125 pg/mL Standard	1200 µL
0	-	1800 µL

- Distribute these standards in wells A1 to E1 of the square well.

If a microplate reader is integrated in the system:

Write and save a protocol in Softmax® for the plate reader.

At the end of the Biomek 2000 method double on the following line: Execute plate reader Protocol: c:\SOFTMAX\XXX.PRO enter the path to the protocol file in the upper window and the path to the data file in the lower window.

A good solution to retrieve data is to enter: c:\DATA*.DAT as the data path. The files will be automatically named as follow: YYMMDDXX.DAT where XX is a number from 01 to 99.

If there is no microplate reader integrated in the system:

Unselect the plate reader from the Setup of the method and delete the following lines at the end of the method:

Gripper move B5, BCIOT EIA to A1

Execute plate reader Protocol: c:\SOFTMAX\XXX.PRO

Gripper move A1, BCIOT EIA to B5

The protocol will be completed, including the addition of the Stop solution but the absorbance measurement will be done by the user on a stand-alone microplate reader.