ELISA for quantification of monocyte chemoattractant protein-1 (MCP-1/CCL2) in human serum or plasma

Angel A Justiz-Vaillant¹, Belkis Ferrer-Cosme²

¹University of the West Indies St. Augustine;
²"Saturnino Lora Torres" Provincial Teaching Clinical Surgical Hospital.
Cuba

University of the West Indies  angel.vaillant@sta.uwi.edu

Angel A Justiz-Vaillant
University of the West Indies St. Augustine

ABSTRACT

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family, and it is a potent chemotactic factor for monocytes.
1. An anti-human monocyte chemoattractant protein-1 (MCP-1/CCL2) coating antibody is adsorbed onto the microwells by incubation overnight at 4°C with carbonate-bicarbonate buffer.

2. Add 50 µl of human serum or plasma into the wells. Human MCP-1/CCL2 present in the serum sample binds to antibodies adsorbed into the microwells.

3. The microplate is blocked with 3% non-fat milk-PBS buffer and later wash to remove unbound proteins.

4. Fifty (50) µl of biotin-conjugated anti-monocyte chemoattractant protein-1 (MCP-1/CCL2) antibody is added. The optimal dilution must be investigated.

5. The microplate is rewashed with PBS-Tween 20 buffer, pH 7.4.

6. One hundred µl of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-human MCP-1/CCL2 antibody.

7. The plate is washed following incubation to remove the unbound Streptavidin-HRP conjugate.
Add 100 µl of 3,3',5,5'- tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.

Incubate the microwells in the dark for 15 min.

A colored product is formed in proportion to the quantity of MCP-1/CCL2 present in the sample or standard.

The reaction is terminated by addition of 100 µl 3M H2SO4 and the absorbance is measured at 450 nm.

A standard curve is made from 7 human MCP-1/CCL2 standard dilutions and the human MCP-1/CCL2 sample concentration is determined.

For better results place the microplate on a microplate shaker in every incubation.