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ELISA for measurement of monocyte chemoattractant protein-1 (MCP-1/CCL2) in human serum.

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ABSTRACT

MCP-1 is believed to play an important role in monocyte infiltration into tumor tissues. [1] MCP-1 plays an important role in the pathogenesis of atherosclerosis. There is considerable evidence that supports that monocytes containing MCPs and macrophages influence the growth of other cell types within the atherosclerotic lesion. [2]

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1 An anti-human 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. Human MCP-1/CCL2 present in the serum or plasma 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-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-MCP-1/CCL2 antibody. The optimal dilution of this conjugate must be investigated.

7 The plate is washed following incubation to remove the unbound Streptavidin-HRP.

8 Add 100 µl of 3',3',5',5'- tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.

9 Incubate the microwells in the dark for 20 min.

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

11 The reaction is terminated by addition of 100 µl 3M H_2SO_4 and the absorbance is measured at 450 nm.

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

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

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