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ELISA for quantification of granulocyte macrophage-colony stimulating factor (GM-CSF) in tissue culture supernatant, human serum or plasma.

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Protocol status: In development
We are still developing and optimizing this protocol

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Disclaimer

Abstract

Granulocyte macrophage-colony stimulating factor (GM-CSF) is a monomeric glycoprotein. It is a cytokine secreted by macrophages, T cells, natural killer cells, mast cells, endothelial cells and fibroblasts. It acts as a growth factor. [1]

Reference

1. Egea L, Hirata Y, Kagnoff MF. GM-CSF: a role in immune and inflammatory reactions in the intestine. Expert Rev Gastroenterol Hepatol. 2010 Dec;4(6):723-31. doi: 10.1586/egh.10.73. PMID: 21108592; PMCID: PMC3291482.

1 An anti-human granulocyte macrophage-colony stimulating factor (GM-CSF) 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. GM-CSF 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-GM-CSF 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-GM-CSF antibody.

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

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

9 Incubate the microwells in the dark for 15 min.

10 A colored product is formed in proportion to the quantity of GM-CSF 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 GM-CSF standard dilutions and the human GM-CSF sample concentration is determined.

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