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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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## 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<sub>2</sub>SO<sub>4</sub> 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.