



Sep 04, 2020

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

DOI

dx.doi.org/10.17504/protocols.io.bktykwpw

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 In...

angel.vaillant@sta.uwi.e...



Angel A Justiz-Vaillant

University of the West Indies St. Augustine

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bktykwpw>

Protocol Citation: Angel A Justiz-Vaillant, Belkis Ferrer-Cosme 2020. ELISA for quantification of granulocyte macrophage-colony stimulating factor (GM-CSF) in tissue culture supernatant, human serum or plasma.. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bktykwpw>



License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development

We are still developing and optimizing this protocol

Created: September 04, 2020

Last Modified: September 04, 2020

Protocol Integer ID: 41560

Keywords: granulocyte macrophage, cytokine, macrophage, granulocyte, stimulating factor, monomeric glycoprotein, natural killer cell, inflammatory reactions in the intestine, mast cell, human serum, inflammatory reaction, gm, csf, tissue culture supernatant, elisa for quantification, endothelial cell

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.

Troubleshooting

- 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 H₂SO₄ 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.