Aug 23, 2020

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

Angel A Justiz-Vaillant[1], Belkis Ferrer-Cosme[2]

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

1 Works for me dx.doi.org/10.17504/protocols.io.bj7akrie

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

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

ABSTRACT

The granulocyte-macrophage colony-stimulating factor (GM-CSF) was initially classified as a hematopoietic growth factor. Instead, in inflammation, GM-CSF serves as a network of communication between tissue-invading lymphocytes and myeloid cells. Even though lymphocytes are in all likelihood the instigators of chronic inflammatory disease, GM-CSF-activated phagocytes are equipped to cause tissue damage [1].

Reference

1. Becher B, Tugues S, Greter M. GM-CSF: From Growth Factor to Central Mediator of Tissue Inflammation. Immunity. 2016;45(5):963-973. doi:10.1016/j.immuni.2016.10.026

DOI

dx.doi.org/10.17504/protocols.io.bj7akrie

PROTOCOL CITATION

Angel A Justiz-Vaillant, Belkis Ferrer-Cosme 2020. ELISA for quantification of granulocyte-macrophage colony-stimulating factor (GM-CSF) in human serum or plasma.. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj7akrie

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

CREATED

Aug 23, 2020

LAST MODIFIED

Aug 23, 2020

PROTOCOL INTEGER ID

40898

1 An anti-human 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. Human GM-CSF present in the serum or plasma binds to antibodies adsorbed into the microwells.

Citation: Angel A Justiz-Vaillant, Belkis Ferrer-Cosme (08/23/2020). ELISA for quantification of granulocyte-macrophage colony-stimulating factor (GM-CSF) in human serum or plasma.. https://dx.doi.org/10.17504/protocols.io.bj7akrie

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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. 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 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.

Citation: Angel A Justiz-Vaillant, Belkis Ferrer-Cosme (08/23/2020). ELISA for quantification of granulocyte-macrophage colony-stimulating factor (GM-CSF) in human serum or plasma.. https://dx.doi.org/10.17504/protocols.io.bj7akrie

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited