ELISA for quantification of Granulocyte-colony stimulator factor (G-CSF) in human serum or plasma

Angel A Justiz-Vaillant¹, Belkis Ferrer-Cosme²

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

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

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

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1 An anti-human granulocyte-colony stimulator factor (G-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. Human G-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-G-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-human G-CSF antibody.

7 The plate is washed following incubation to remove the unbound Streptavidin-HRP.
Add 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.

Incubate the microwells in the dark for 15 min.

A colored product is formed in proportion to the quantity of G-CSF present in the sample or standard.

The reaction is terminated by addition of 100 µl 3M H2SO4 and the absorbance is measured at 450 nm.

A standard curve is made from 7 human G-CSF standard dilutions and the human G-CSF sample concentration is determined.

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