Sep 03, 2020

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

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In Development dx.doi.org/10.17504/protocols.io.bks2kwge

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DOI

dx.doi.org/10.17504/protocols.io.bks2kwge

PROTOCOL CITATION

Angel A Justiz-Vaillant, Belkis Ferrer-Cosme 2020. ELISA for quantification of Granulocyte-colony stimulator factor (G-CSF) in human serum or plasma. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bks2kwge

CREATED

Sep 03, 2020

LAST MODIFIED

Sep 03, 2020

PROTOCOL INTEGER ID

41530

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.

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 G-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 G-CSF standard dilutions and the human G-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 (09/03/2020). ELISA for quantification of Granulocyte-colony stimulator factor (G-CSF) in human serum or plasma. https://dx.doi.org/10.17504/protocols.io.bks2kwge