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ELISA for quantification of human C3 in 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.bj72krqe

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

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

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1 An anti-human C3 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 C3 present in the serum or plasma 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-C3 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-C3 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 C3 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 C3 standard dilutions and the human C3 sample concentration is determined.

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