ELISA for quantification of human immunoglobulin G (IgG) in serum or plasma.

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1 Works for me

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ABSTRACT

IgG is a monomer with an approximate molecular weight of 146 Kd and a serum concentration of 9.0 mg/mL. It is synthesized mostly in the secondary immune response to pathogens. IgG can activate the classical pathway of the complement system, and it also is highly protective. The four subclasses of IgG include IgG1, IgG2, IgG3, and IgG4. IgG crosses the placentae, protecting the neonate from infectious diseases. [1]

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1 An anti-human IgG 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 IgG 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-IgG 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-IgG 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 IgG 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 IgG standard dilutions and the human IgG sample concentration is determined.

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

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