ELISA for quantification of human immunoglobulin A (IgA) in serum or plasma.

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1 Works for me

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ABSTRACT

IgA appears in 2 different molecular structures: monomeric (serum) and dimeric structure (secretory). The serum IgA has a molecular weight of 160 Kd and a serum concentration of 3 mg/mL. Secretory IgA (sIgA) has a molecular weight of 385 Kd and a mean serum concentration of 0.05 mg/mL. It appears in mucosa membranes as a dimer (with J chain when secreted) and protects the epithelial surfaces of the respiratory, digestive, and genitourinary system. IgA possesses a secretory component that prevents its enzymatic digestion. It activates the alternative pathway of activation of the complement system. [1]

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

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

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