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

Angel A Justiz-Vaillant¹, Belkis Ferrer-Cosme²

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

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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PROTOCOL

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.
Fifty (50) µl of biotin-conjugated anti-IgA antibody is added. The optimal dilution must be investigated.

The microplate is rewashed with PBS-Tween 20 buffer, pH 7.4.

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.

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 20 min.

A colored product is formed in proportion to the quantity of IgA 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 IgA standard dilutions and the human IgA sample concentration is determined.

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

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