ELISA for quantification of human immunoglobulin E (IgE) 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

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

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

ABSTRACT
IgE is a monomer. It has a molecular weight of 188 Kd and a serum concentration of 0.00005 mg/mL. It protects against parasites and also binds to high-affinity receptors on mast cells and basophils causing allergic reactions. [1]

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1 An anti-human IgE 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 IgE 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-IgE antibody is added. The optimal dilution must be investigated.

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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-IgE 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 human IgE 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 IgE standard dilutions and the human IgE sample concentration is determined.

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