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ELISA for quantification of IL-13 in human serum.

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We are still developing and optimizing this protocol

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Abstract

Interleukins (IL) are a type of cytokine first thought to be expressed by leukocytes alone but have later been found to be produced by many other body cells. They play essential roles in the activation and differentiation of immune cells, as well as proliferation, maturation, migration, and adhesion. They also have pro-inflammatory and anti-inflammatory properties. The primary function of interleukins is, therefore, to modulate growth, differentiation, and activation during inflammatory and immune responses. Interleukins consist of a large group of proteins that can elicit many reactions in cells and tissues by binding to high-affinity receptors in cell surfaces.

CD4+T cells (Th2), NKT cells and mast cells synthesize IL-13. It acts on monocytes, fibroblasts, epithelial cells and B cells. The IL-13 significant effects are B-cell growth and differentiation, stimulates isotype switching to IgE. It causes increased mucus production by epithelial cells, increased collagen synthesis by fibroblasts and inhibits pro-inflammatory cytokine production. Also, IL-13 works together with IL-4 in producing biologic effects associated with allergic inflammation and in defense against parasites. **[1]**

Reference

1. Justiz Vaillant AA, Qurie A. Interleukin. In:*StatPearls*. Treasure Island (FL): StatPearls Publishing; June 12, 2019.

Troubleshooting

1 An anti-human IL-13 coating antibody is adsorbed onto microwells by incubation overnight at 4°C.

2 Add 50 µl of human serum. Human IL-13 present in the serum sample binds to antibodies adsorbed to 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-human IL-13 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-human IL-13 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) to each well.

9 Incubate the microwells in the dark for 15 min.

10 A colored product is formed in proportion to the quantity of human IL-13 present in the sample or standard.

11 The reaction is terminated by addition of 100 µl 3M H_2SO_4 and absorbance is measured at 450 nm.

12 A standard curve is made from 7 human IL-13 standard dilutions and the human IL-13 sample concentration is determined.

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