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ELISA for quantification of IL-10 in human serum.

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Protocol status: Working

We use this protocol and it's working

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Abstract

Interleukin-10 is a pleiotropic cytokine playing a critical role as a regulator of myeloid and lymphoid cell function. Due to the ability of IL-10 to blocking cytokine synthesis and many accessory cell functions of antigen-presenting cells such as macrophages this cytokine is a potent suppressor of the effector functions of macrophages, T-cells and NK cells. IL-10 also participates in regulating proliferation and differentiation of B-lymphocytes, mast cells and thymocytes [1].

Reference

1. Ouyang W, O'Garra A. IL-10 Family Cytokines IL-10 and IL-22: from Basic Science to Clinical Translation. *Immunity*. 2019;50(4):871-891. doi:10.1016/j.immuni.2019.03.020

Materials

MATERIALS

 IL-10, Interleukin-10, human **Bio Basic Inc.** **Catalog #RC212-21**.SIZE.2ug

- 1 An anti-human IL-10 coating antibody is adsorbed onto microwells by incubation overnight at 4°C.
- 2 Add 50 µl of human serum. Human IL-10 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 washed to remove unbound proteins.
- 4 Fifty (50) µl of biotin-conjugated anti-human IL-10 antibody is added.
- 5 The microplate is rewashed with PBS-Tween buffer.
- 6 One hundred µl of streptavidin-HRP is added and binds to the biotin-conjugated anti-human IL-10 antibody.
- 7 The plate is washed following incubation to remove the unbound Streptavidin-HRP.
- 8 Add 50 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-10 present in the sample or standard.
- 11 The reaction is terminated by addition of 3M H₂SO₄ and absorbance is measured at 450 nm.
- 12 A standard curve is made from 7 human IL-10 standard dilutions and the human IL-10 sample concentration determined.



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