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ELISA for quantification of IL-6 in human serum or plasma.

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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

Troubleshooting

1 An anti-human IL-6 coating antibody is adsorbed onto microwells by incubation overnight at 4°C.

2 Add 50 µl of human serum. Human IL-6 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 wasth o remove unbound proteins.

4 Fifty (50) µl of biotin-conjugated anti-human IL-6 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-6 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-6 present in the sample or standard.

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

12 A standard curve is made from 7 human IL-6 standard dilutions and the human IL-6 sample concentration determined.

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