ELISA for quantification of tumor necrosis factor alpha (TNF-α) in human serum or plasma.

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Aug 23, 2020

1 Works for me

dx.doi.org/10.17504/protocols.io.bj69krh6

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ABSTRACT

Tumor necrosis factor (TNF) is a cell signaling cytokine involved in systemic inflammation. It is one of the proteins that make up the acute phase reaction [1].

Reference

1. Pasquereau S, Kumar A, Herbein G. Targeting TNF and TNF Receptor Pathway in HIV-1 Infection: from Immune Activation to Viral Reservoirs. *Viruses*. 2017;9(4):64. Published 2017 Mar 30. doi:10.3390/v9040064

DOI

dx.doi.org/10.17504/protocols.io.bj69krh6

PROTOCOL CITATION

Angel A Justiz-Vaillant, Belkis Ferrer-Cosme 2020. ELISA for quantification of tumor necrosis factor alpha (TNF-α) in human serum or plasma.. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bj69krh6

CREATED

Aug 23, 2020

LAST MODIFIED

Aug 23, 2020

PROTOCOL INTEGER ID

40897

1 An anti-human TNF-α 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 TNF-α 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-TNF-α 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-TNF-α 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) into each well.

9 Incubate the microwells in the dark for 20 min.

10 A colored product is formed in proportion to the quantity of TNF-α present in the sample or standard.

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

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

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

Citation: Angel A Justiz-Vaillant, Belkis Ferrer-Cosme (08/23/2020). ELISA for quantification of tumor necrosis factor alpha (TNF-ÃÂ±) in human serum or plasma.. https://dx.doi.org/10.17504/protocols.io.bj69krh6