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# ELISA for quantification of interferon gamma (IFN- $\gamma$ ) in human serum or plasma.

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## ABSTRACT

IFN- $\gamma$  is a key player in driving cellular immunity. It is capable of orchestrating numerous and vital protective functions to enhance immune responses in cancers and infectious diseases. It exhibits its immunomodulatory effects by enhancing antigen processing and presentation,, inducing an anti-viral state, increasing leukocyte trafficking, boosting the anti-microbial properties of immune cells and by affecting cellular proliferation and apoptosis. [1]

## Reference

Kak G, Raza M, Tiwari BK. Interferon-gamma (IFN- $\gamma$ ): Exploring its implications in infectious diseases. *Biomol Concepts*. 2018;9(1):64-79. Published 2018 May 30. doi:10.1515/bmc-2018-0007

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- 1 An anti-human IFN- $\gamma$  coating antibody is adsorbed onto the microwells by incubation overnight at 4°C with carbonate-bicarbonate buffer.
- 2 Add 50  $\mu$ l of human serum or plasma. Human IFN- $\gamma$  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-IFN-γ 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-IFN-γ 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 IFN-γ present in the sample or standard.
- 11 The reaction is terminated by addition of 100 µl 3M H<sub>2</sub>SO<sub>4</sub> and the absorbance is measured at 450 nm.
- 12 A standard curve is made from 7 human IFN-γ standard dilutions and the human IFN-γ sample concentration is determined.
- 13 For better results place the microplate on a microplate shaker in every incubation.