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# ELISA for quantification of human immunoglobulin M (IgM) in serum or plasma.

Angel A Justiz-Vaillant<sup>1</sup>, Belkis Ferrer-Cosme<sup>2</sup><sup>1</sup>University of the West Indies St. Augustine; <sup>2</sup>Saturnino Lora Torres' Provincial Teaching Clinical Surgical Hospital. Cuba**1** Works for me [dx.doi.org/10.17504/protocols.io.bj7rkrm6](https://dx.doi.org/10.17504/protocols.io.bj7rkrm6)[University of the West Indies](#) [angel.vaillant@sta.uwi.edu](mailto:angel.vaillant@sta.uwi.edu)Angel Justiz-Vaillant  
University of the West Indies St. Augustine

## 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 IgM 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 IgM 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)  $\mu$ l of biotin-conjugated anti-IgM 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  $\mu$ l of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-IgM 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  $\mu$ l 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 IgM present in the sample or standard.
- 11 The reaction is terminated by addition of 100  $\mu$ l 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 IgM standard dilutions and the human IgM sample concentration is determined.
- 13 For better results place the microplate on a microplate shaker in every incubation.