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# ELISA for quantification of CXC motif chemokine ligand 2 (CXCL2) in human serum or plasma.

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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

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- 1 An anti-human CXCL2 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 CXCL2 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-CXCL2 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-CXCL2 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 CXCL2 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 CXCL2 standard dilutions and the human CXCL2 sample concentration is determined.
- 13 For better results place the microplate on a microplate shaker in every incubation.