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ELISA for measurement of platelet-activating factor (PAF) in serum.

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Protocol status: In development
We are still developing and optimizing this protocol

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1 An anti-human PAF 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 PAF 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-PAF 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-PAF 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 PAF 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 PAF standard dilutions and the human PAF sample concentration is determined.

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