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ELISA for quantification of Vascular endothelial growth factor A (VEGFA) in cell culture supernatant, human serum or plasma

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- 1 An anti-vascular endothelial growth factor A (VEGFA) coating antibody is adsorbed onto the microwells by incubation overnight at 4°C with carbonate-bicarbonate buffer.

Add 50 µl of human serum. Human VEGFA present in the serum sample binds to antibodies adsorbed into the

- 2 microwells.
- 3 The microplate is blocked with 3% non-fat milk-PBS buffer and later wash to remove unbound proteins.
- 4 Fifty (50) μ l of biotin-conjugated anti-vascular endothelial growth factor A 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 μ l of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-human VEGFA antibody.
- 7 The plate is washed following incubation to remove the unbound Streptavidin-HRP conjugate.
- 8 Add 100 μ l of 3,3',5,5'- tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.
- 9 Incubate the microwells in the dark for 15 min.
- 10 A colored product is formed in proportion to the quantity of VEGFA present in the sample or standard.
- 11 The reaction is terminated by addition of 100 μ l 3M H₂SO₄ and the absorbance is measured at 450 nm.
- 12 A standard curve is made from 7 human VEGFA standard dilutions and the human VEGFA sample concentration is determined.
- 13 For better results place the microplate on a microplate shaker in every incubation.