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

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

The microplate is blocked with 3% non-fat milk-PBS buffer and later wash to remove unbound proteins.

Fifty (50) µl of biotin-conjugated anti-vascular endothelial growth factor A antibody is added. The optimal dilution must be investigated.

The microplate is rewashed with PBS-Tween 20 buffer, pH 7.4.

One hundred µl of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-human VEGFA antibody.

The plate is washed following incubation to remove the unbound Streptavidin-HRP conjugate.
Add 100 µl of 3,3',5,5'-tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.

Incubate the microwells in the dark for 15 min.

A colored product is formed in proportion to the quantity of VEGFA present in the sample or standard.

The reaction is terminated by addition of 100 µl 3M H2SO4 and the absorbance is measured at 450 nm.

A standard curve is made from 7 human VEGFA standard dilutions and the human VEGFA sample concentration is determined.

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