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

Angel A Justiz-Vaillant[1], Belkis Ferrer-Cosme[2]

[1]University of the West Indies St. Augustine; [2]"Saturnino Lora Torres" Provincial Teaching Clinical Surgical Hospital. Cuba

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University of the West Indies angel.vaillant@sta.uwi.edu

Angel Justiz-Vaillant
University of the West Indies St. Augustine

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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

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2 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-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 µl 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 µl 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 µl 3M H_2SO_4 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.

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