ELISA for quantification of RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) in human serum or plasma.

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ABSTRACT

RANTES (Regulated on Activation, Normal T cell Expressed and Secreted) also known as CCL5 is a chemotactic cytokine for T cells, basophils, and eosinophils. RANTES also acts as a recruitment signal for immune cells into inflammatory sites.

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1 An anti-human RANTES 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 RANTES (CCL5) 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.
Fifty (50) µl of biotin-conjugated anti-RANTES 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-RANTES antibody. The optimal dilution of this conjugate must be investigated.

The plate is washed following incubation to remove the unbound Streptavidin-HRP.

Add 100 µl of 3',3',5',5'-tetramethylbenzidine (TMB; Sigma-Aldrich) into each well.

Incubate the microwells in the dark for 20 min.

A colored product is formed in proportion to the quantity of RANTES (CCL5) 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 RANTES standard dilutions and the human RANTES sample concentration is determined.

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

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