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Enzyme linked immunosorbent assay (ELISA) for determining the serum concentration of IL-33 in humans.

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1 Ninety-six well ELISA plates are coated with monoclonal anti-human antibodies to interleukin-33 (IL-33).

2 Patient serum samples are added to the plates.

3 The plate is incubate x 1.30 hour at RT.

4 The plate is washed 4 times with PBS-tween buffer.

5 The wells are incubated with a biotin conjugated anti-human IL-33 for 1.30 hour at RT.

6 The plate is washed again as above.

7 Add to the plate a peroxidase-labeled streptavidin conjugate and incubate it for 1 hour at RT.

8 After a further washing procedure a substrate solution reactive is added and allowed to produced a colored reaction in positive controls.

9 The level of IL-33 in the sample is proportional to the colored product developed.

10 The addition of 3M H2SO4 stops the reaction.

11 The absorbance is measured at 450 nm.

12 The IL-33 concentration can be calculated by generating an standard curve using lineal regression.

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