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

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**Protocol status:** Working

**We use this protocol and it's working**

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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 H<sub>2</sub>SO<sub>4</sub> 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.