



Aug 23, 2020

# ELISA for quantification of human C4 in serum or plasma.

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In Development

[dx.doi.org/10.17504/protocols.io.bj75krq6](https://dx.doi.org/10.17504/protocols.io.bj75krq6)

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DOI

[dx.doi.org/10.17504/protocols.io.bj75krq6](https://dx.doi.org/10.17504/protocols.io.bj75krq6)

## PROTOCOL CITATION

Angel A Justiz-Vaillant, Belkis Ferrer-Cosme 2020. ELISA for quantification of human C4 in serum or plasma.. **protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.bj75krq6>

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## CREATED

Aug 23, 2020

## LAST MODIFIED

Aug 23, 2020

## PROTOCOL INTEGER ID

40925

- 1 An anti-human C4 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 C4 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.
- 4 Fifty (50) µl of biotin-conjugated anti-C4 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  $\mu$ l of streptavidin-HRP conjugate is added and it binds to the biotin-conjugated anti-C4 antibody. The optimal dilution of this conjugate must be investigated.
- 7 The plate is washed following incubation to remove the unbound Streptavidin-HRP.
- 8 Add 100  $\mu$ l 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 C4 present in the sample or standard.
- 11 The reaction is terminated by addition of 100  $\mu$ l 3M H<sub>2</sub>SO<sub>4</sub> and the absorbance is measured at 450 nm.
- 12 A standard curve is made from 7 human C4 standard dilutions and the human C4 sample concentration is determined.