

Protocol 507_Total Remicade/Infliximab and anti-Remicade/ Infliximab antibody ELISAs

Reagents

Human TNF (R&D). Resuspended at 10mg/ml in RPMI and stored in frozen aliquots

Remicade, 100mg resuspended in 10ml PBS. Aliquoted and frozen for long-term storage (avoid repeated freeze-thaw)

HRP-anti-human IgG (Bethyl). Use 1:5000

Bt-anti-human IgG2, Bt-anti-human IgG3, Bt-anti-human IgG4 (Invitrogen) – use 1:200

HRP-Streptavidin (Biolegend) – use 1:1000

ELISA coating buffer – Carbonate pH 9 0.1M NaHCO₃ (3.18g Na₂CO₃ + 5.88g NaHCO₃ made up to 1000ml with water).

ELISA substrate buffer - 0.1M NaH₂PO₄, pH 4 (13.8g NaH₂PO₄ made up to 1000ml)

ELISA block: PBS 2% BSA

ABTS

H₂O₂

Method – total active Remicade quantification

1. Coat plates with 50ng/ml Purified human TNF in 50µl coating buffer
2. Incubate 3hrs to overnight at 4°C
3. Wash 3x with ELISA wash buffer
4. Add 200µl per well ELISA blocking buffer incubate 4°C for 3hrs – overnight
5. Add Remicade standards (start at 20ng/ml top concentration – 1:500000 – 3x dilution – diluted in ELISA blocking buffer)
6. Add plasma/serum diluted 1:10 in blocking buffer and diluted 3-fold down plate.
7. Incubate 3h to overnight at 4°C
8. Wash 6x with ELISA wash buffer
9. Add 50µl HRP-anti-mouse IgG 1:1000 or HRP-anti-human IgG (will also detect Cimzia and Humira) 1:5000 in ELISA block per well
10. Incubate 1hr rm temp
11. Wash 6x with ELISA wash buffer

12. Add 100µl per well of ELISA substrate (e. Make up 10ml substrate per plate (10ml substrate buffer, 1mg ABTS, 5µl H₂O₂ – do not allow this to sit for more than about 10mins after the H₂O₂ has been added)
13. Incubate at rm temp for 30' to 1hr

14. Read A405 nm

15. Method – anti-Remicade IgG quantification (NB Remicade is an IgG1)

16. Coat plates with 10µg/ml Remicade in 50µl coating buffer

17. Incubate 3hrs to overnight at 4°C

18. Wash 3x with ELISA wash buffer

19. Add 200µl per well ELISA blocking buffer incubate 4°C for 3hrs – overnight

20. Add plasma/serum diluted 1:10 in blocking buffer and diluted 3-fold down plate.

21. Incubate 3h to overnight at 4°C

22. Wash 6x with ELISA wash buffer

23. Add 50µl Bt-anti-hIgG2, hIgG3 and hIgG4 mixed 1:2000 in ELISA block per well

24. Incubate 1hr rm temp

25. Wash 6x with ELISA wash buffer

26. Add 50µl per well of HRP-SA 1:1000 in ELISA block

27. Incubate 1hr rm temp

28. Wash 6x with ELISA wash buffer

29. Add 100µl per well of ELISA substrate (e. Make up 10ml substrate per plate (10ml substrate buffer, 1mg ABTS, 5µl H₂O₂ – do not allow this to sit for more than about 10mins after the H₂O₂ has been added)

30. Incubate at rm temp for 30' to 1hr

31. Read A405 nm