

Quantitative TNF-alpha Sandwich ELISA

INTRODUCTION

ELISA (Enzyme Linked ImmunoSorbent Assay) provides a highly sensitive and precise method for the estimation of biological parameters. The method has the advantage of rapidly analyzing large numbers of samples. ELISA is used for detection, identification, quantification of a particular protein, as well as for discrimination (i.e. subtyping) between proteins. The Anti-TNF-alpha Affibody[®] molecule is a specific affinity ligand that efficiently captures human TNF-alpha and is well suited as capture reagent in a sandwich ELISA.

RESULTS

QUANTITATIVE ELISA

The Anti-TNF-alpha Affibody[®] molecule can be used as capture reagent in a sandwich ELISA in combination with a mouse anti-human TNF-alpha monoclonal antibody as the detection reagent. Titration of TNF-alpha gives a sigmoid curve with a sensitivity of 60 pg TNFalpha/ml (defined as two times background value) and a measurement interval between 100 and 1000 pg/ml.

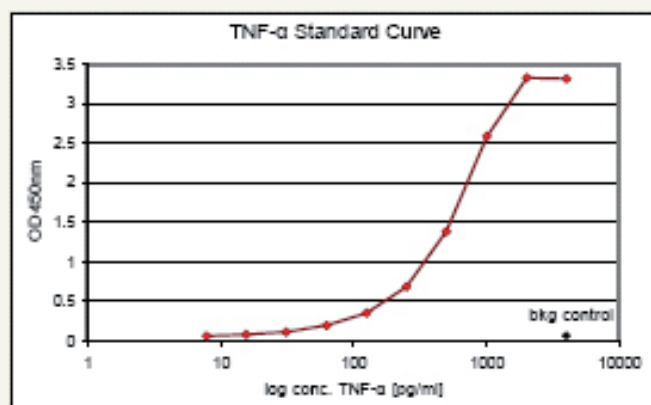


Fig. 1. Standard TNF-alpha was titrated on Anti-TNF-alpha Affibody[®] molecule coated plates with a sensitivity of 60 pg/ml.

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MATERIALS AND BUFFERS REQUIRED

Coating ligand: Anti-TNF-alpha Affibody[®] molecule (Affibody cat no 10.0841.01.0010)

Coating buffer: 15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6

ELISA plates: 96-well, flat bottomed, high protein binding plates (Costar cat no 9018)

PBST: 2.68 mM KCl, 1.47 mM KH₂PO₄, 137 mM NaCl, 8.1 mM Na₂HPO₄, pH 7.4, 0.05%, Tween 20

Blocking buffer: PBS + 0.5% casein

TNF-alpha: (Research Diagnostics cat no RDI-301X)

Anti-TNF-alpha antibody: Mouse anti-TNF-alpha antibody (Abcam cat no ab9813)

HRP-conjugate: Anti-mouse labeled polymer-HRP (EnVision System, Dako Cytomation cat no K4000)

Substrate: ImmunoPure[®] TMB Substrate Kit (Pierce cat no 34021)

Stop buffer: 2 M H₂SO₄

PROTOCOL

1. Dilute the Affibody[®] molecule in coating buffer to a final concentration of 1 µg/ml. Coat a flatbottomed, high protein binding 96-well plate by adding 100 µl of the diluted Affibody[®] molecule per well.
2. Cover the plate with an adhesive plastic and incubate at +4°C over night.
3. Remove the coating solution and wash the plate twice with deionized water. Use an automatic ELISA washer or flick the plate over a sink. The remaining drops are removed by dabbing the plate on a paper towel.
4. Block the remaining protein binding sites by incubation with blocking buffer. Add 200 µl per well, cover the plate with plastics and incubate for 1 hour at room temperature.
5. Empty the plate without washing.
6. Add 100 µl per well of sample and negative control diluted in PBST. Use purified TNF-alpha as standard. The dilutions are to be determined by the user (see application note for information about the measurement interval).
7. Cover the plate with plastics and incubate for 1 hour at room temperature.

8. Wash the plate four times with PBST.
9. Dilute the mouse anti-TNF-alpha antibody to a final concentration of 1 µg/ml in PBST. This antibody works well in pair with the Anti-TNF-alpha Affibody[®] molecule. Any other anti-TNF-alpha antibody has to be tested by the user.
10. Add 100 µl/well, cover the plate with plastics and incubate for 1 hour at room temperature.
11. Wash the plate 4 times with PBST.
12. Dilute anti-mouse labeled polymer-HRP 1:50 in PBST. Add 100 µl/well, cover the plate with plastics and incubate for 1 hour at room temperature.
13. Wash the plate 4 times with PBST.
14. Add 100 µl per well of ImmunoPure[®] TMB Substrate Kit prepared as described by the manufacturer. Stop the reaction after 30 minutes with stop buffer.
15. Measure the absorbance at 450 nm using a microtiter-plate spectrophotometer.
16. Plot the absorbance values against the concentration to obtain a standard curve.
17. Use the standard curve to determine the concentration of TNF-alpha in the sample.

LIMITATIONS

Warranty: Affibody[®] products are warranted to meet stated product specifications and to confirm to label descriptions when used and stored properly. Unless otherwise stated, this warranty is limited to one year from date of sales for products used, handled and stored according to Affibody AB's instructions. Affibody AB's sole liability is limited to replacement of the product or refund of the purchase price. Affibody[®] products are supplied for research use only. They are not intended for medicinal, diagnostic or therapeutic use. Affibody[®] products may not be resold, modified for resale or used to manufacture commercial products without prior written approval from Affibody AB.

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