Quantitative Transthyretin 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 or quantification of a particular protein, as well as for discrimination (i.e. subtyping) between proteins.

The Anti-Transthyretin Affibody® molecule is a specific affinity ligand that binds to human transthyretin (prealbumin) and is well suited as capture reagent in a sandwich ELISA.

RESULTS QUANTITATIVE ELISA

The Anti-Transthyretin Affibody® molecule can be used as capture reagent in a sandwich ELISA in combination with a goat anti-transthyretin antibody as the detection reagent. Titration of transthyretin gives a sigmoid curve with a sensitivity of 4 ng transthyretin/ml (defined as two times background value).

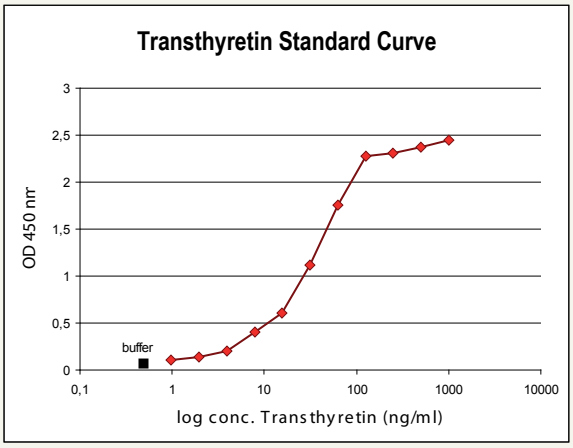

Fig. 1. Transthyretin standard curve. Standard transthyretin was titrated on Anti-Transthyretin Affibody® molecule coated plates with a sensitivity of 4 ng/ml.

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MATERIALS AND BUFFERS REQUIRED

Coating ligand: Anti-Transthyretin Affibody® molecule: (Affibody cat no 10.1393.01.0005)
Coating buffer: 15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6
ELISA plates: 96-well, flat bottomed, high protein binding plates (Costar cat no 3690)
PBST: 2.68 mM KCl, 1.47 mM KH_2PO_4, 137 mM NaCl, 8.1 mM Na_2HPO_4, pH 7.4, 0.05%, Tween 20
Blocking buffer: PBS + 0.5% casein (Sigma 8654)
Transthyretin: Sigma Prealbumin from human plasma P1742-1MG
Anti-transthyretin ab: Goat anti-prealbumin antibody (Abcam cat no ab15007)
HRP conjugate: Rabbit anti-goat IgG (Dako cat no P0449)
Substrate: ImmunoPure® TMB Substrate Kit (Pierce cat no 34021)
Stop buffer: 2 M H_2SO_4

PROTOCOL

1. Dilute the Affibody® molecule in coating buffer to a final concentration of 5.0 µg/ml. Coat a flat-bottomed, high protein binding 96-well plate by adding 50 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 can be removed by dabbing the plate on a paper towel.
4. Block the remaining protein binding sites by incubation with blocking buffer. Add 100 µl per well, cover the plate with plastics and incubate for 1 hour at room temperature.
5. Empty the plate without washing.
6. Add 50 µl per well of sample and negative control diluted in PBST. Use purified transthyretin as standard. The dilutions should be determined by the user (see application note for information about the concentration interval).
7. Cover the plate with plastics and incubate for 1 hour at room temperature.
8. Wash the plate 4 times with PBST.
9. Dilute the goat anti-transthyretin antibody to a final concentration 1:100 000 in PBST. This antibody works well in pair with the Anti-Transthyretin Affibody® molecule. Any other anti-transthyretin antibody has to be tested by the user.
10. Add 50 µl antibody per well, cover the plate with plastics and incubate for 1 hour at room temperature.
11. Wash the plate 4 times with PBST.
12. Dilute HRP conjugated rabbit anti-goat IgG 1:10 000 in PBST. Add 50 µl per well, cover the plate with plastics and incubate for 1 hour at room temperature.
13. Wash the plate 4 times with PBST.
14. Add 50 µl per well of ImmunoPure® TMB Substrate Kit prepared as described by the manufacturer. Stop the reaction after 15 minutes with stop buffer, 50 µl per well.
15. Measure the absorbance at 450 nm using a microtiter-plate spectrophotometer.
16. Plot OD values against the concentration to obtain a standard curve.
17. Use the standard curve to determine the concentration of transthyretin 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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