

Quantitative HSA 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-HSA Affibody[®] molecule is a specific affinity ligand that binds to human HSA and is well suited as capture reagent in a sandwich ELISA.

RESULTS QUANTITATIVE ELISA

The Anti-HSA Affibody[®] molecule can be used as capture reagent in a sandwich ELISA in combination with a rabbit anti-HSA antibody as the detection reagent. Titration of HSA gives a sigmoid curve with a sensitivity of 5 ng HSA/ml (defined as two times background value).

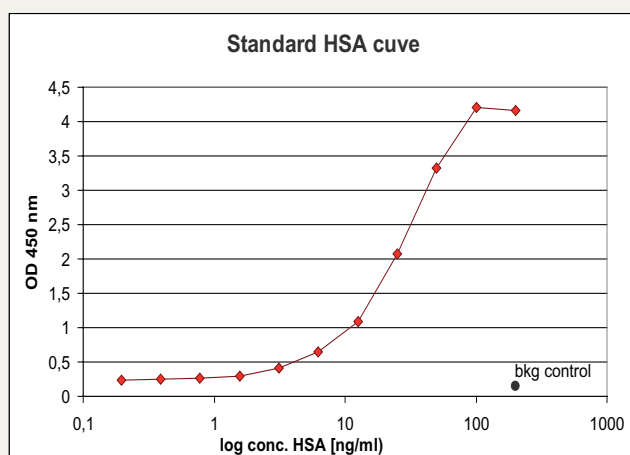


Fig. 1. HSA standard curve. Standard HSA was titrated on Anti-HSA Affibody[®] molecule coated plates with a sensitivity of 5 ng/ml.

SPECIES SPECIFICITY

Sera from six different species, human, mouse, rat, goat, bovine and rabbit were titrated in 3-fold dilution series on Anti-HSA Affibody[®] molecule coated plates. As shown in figure 2, the Anti-HSA Affibody[®] molecule binds with high affinity to human albumin followed by rat and mouse albumin. Low binding was observed to bovine, goat and rabbit albumin. The species discrepancy is dependent on the binding properties of the Anti-HSA Affibody[®] molecule as well as the second step rabbit anti-HSA antibody.

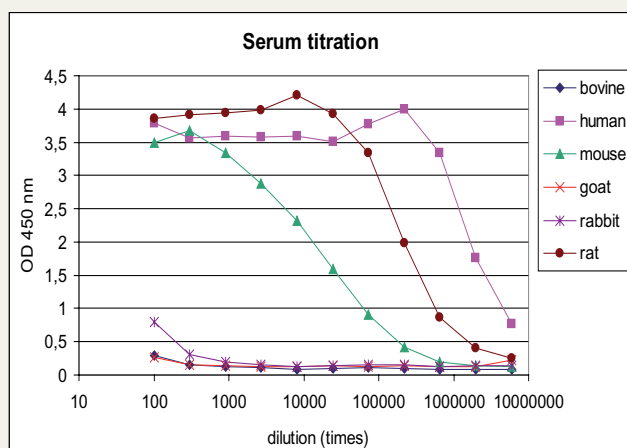


Fig. 2. Sera from different species were titrated on Anti-HSA Affibody[®] molecule

ANALYSIS OF ALBUMIN CONCENTRATION IN DEPLETED SERUM

The remaining HSA in samples from serum, depleted from HSA by passage through an Anti-HSA Affibody[®] molecule coupled column, was analyzed using the Anti-HSA Affibody[®] ELISA. Concentration of HSA in flow through samples from cycle 25, 150, 200 and 300 was analyzed and the percentage of achieved depletion was calculated. The data is presented in table 1.

Depletion cycle	Albumin concentration after depletion (µg/ml)	Achieved albumin depletion (%)
25	10	99.4
150	11	99.4
200	17	99.1
300	16	99.1

Table 1. Depletion efficiency.

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MATERIALS AND BUFFERS REQUIRED

Coating ligand: Anti-HSA Affibody[®] molecule, unconjugated (Affibody cat no 10.0644.01.0010)

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

HSA: (Sigma cat no A3782)

Anti-HSA antibody: Rabbit anti-HSA antibody (Abcam cat no ab1217)

HRP conjugate: Goat anti-rabbit IgG (Dako cat no P0448)

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 10 µg/ml. Coat a flat-bottomed, 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 can be 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 HSA 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 rabbit anti-HSA antibody to a final concentration of 2 µg/ml in PBST. This antibody works well in pair with the Anti-HSA Affibody[®] molecule. Any other anti-HSA antibody has to be tested by the user.
10. Add 100 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 goat-anti rabbit IgG 1:10 000 in PBST. Add 100 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 100 µl per well of ImmunoPure[®] TMB Substrate Kit prepared as described by the manufacturer. Stop the reaction after maximum 30 minutes with stop buffer, 100 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 HSA 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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