Materials provided:

Number of determinations	1x 96
Microplate strips, albumin (bovine) coated	12x 8
Wash buffer, 10fold conc. ✦	40 ml
Diluent, ready to use ✦	100 ml
Standard concentrate, 40960 ng/ml ✦	1 ml
Anti-albumin-Ab., HRP conjugate, 100fold conc. ✦	0.1 ml
TMB substrate, ready to use	12 ml
Stop solution, ready to use (0.5 M sulphuric acid)	12 ml

✦: contains Thimerosal

Assay procedure summary:

A. Preparation

1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Prepare the standard curve from a 1:4-dilution series of standard concentrate with diluent
4. Dilute samples with diluent
5. Dilute freshly HRP conjugate 1:100 with diluent

B. Performance

1. Pipette 50 µl of samples, standards, controls into the wells
2. Immediately add 50 µl of HRP conjugate to each well
3. Incubate for 2 hours at room temperature
4. Wash three times with 300 µl of wash buffer
5. Dispense 100 µl of TMB substrate solution
6. Incubate for 20 minutes at room temperature in the dark
7. Add 100 µl of stop solution
8. Measure absorption at 450 nm

Related products:

ELISA for quantitative determination of albumin in rat urine (Catalog number: 50100)
ELISA for quantitative determination of albumin in mouse urine (Catalog number: 50200)

Contract analysis: determination of BSA (please inquire)
Contract analysis: determination of albumin in rat or mouse urine (please inquire)

Version 01-04/02

INSTRUCTIONS FOR USE

ELISA for Quantitative Determination of Bovine Albumin (BSA)

Catalog No.: 50300

produced for BioClot GmbH

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Introduction

The ALBUMIN(bovine)-ELISA is designed for the quantitative determination of bovine albumin in complex samples. Main application of the assay is to monitor purification of protein preparations (e.g. monoclonal antibodies) if bovine or calf serum albumin has been used in the production process.

This ELISA furthermore allows quantification of albumin in bovine urine to estimate kidney function. When blood is filtered in glomeruli of kidneys, highly molecular substances are held back. Therefore, urine is nearly protein free. In case of kidney damages, small proteins like albumin appear in urine. Albumin therefore serves as a marker in monitoring kidney damages.

Principle of the Assay

The determination of albumin is carried out as direct competitive ELISA. Bovine albumin has been pre-coated onto a microplate. During incubation the binding of an enzyme-linked anti-albumin(bovine)-antibody to the wells is inhibited by albumin from samples/standards. After washing away any unbound antibody, a substrate solution is added to the wells and color develops in proportion to the amount of antibody conjugate. The absorption at 450 nm is reverse proportional to the albumin concentration.

Precautions

Store the kit at 2-8 °C.

For research use only. Not for use in diagnostic procedures.

For in vitro use only.

Do not use the reagents beyond the expiration date marked on box label.

Please read the instructions carefully before using the kit.

Do not mix reagents from different lots.

The conjugate concentrate contains human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious.

Some components of this kit contain Thimerosal, a mercury containing compound. The stop solution contains 0.5 M sulphuric acid. Follow routine precautions for handling hazardous chemicals.

Do not allow the wells to become dry once the assay has begun.

Other supplies required

Deionized or distilled water
Graduated cylinder
Micropipettes, multipipette
Microplate reader

Preparation of reagents and samples

- Bring all reagents to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.

- The microplate strips are ready to use. Remove excess strips (breakable) from the frame, reseal in the plastic bag with the desiccant and store at 2-8 °C

- Dilute the wash buffer with deionized or distilled water **1:10** (e. g. 40 ml + 360 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Dilute the HRP conjugate with diluent **1:100** (e. g. 50 µl + 4950 µl diluent). The required amount of conjugate solution should be prepared freshly.

- Use the Standard concentrate to produce a 1:4-dilution series with diluent (e. g. 100 µl + 300 µl diluent):

standard	preparation	conc. ng/ml
S 7	standard conc. undiluted	40,960
S 6	S 7 1:4 diluted	10,240
S 5	S 6 1:4 diluted	2,560
S 4	S 5 1:4 diluted	640
S 3	S 4 1:4 diluted	160
S 2	S 3 1:4 diluted	40
S 1	S 2 1:4 diluted	10

- Dilute the samples with diluent. To exclude matrix effects the dilution factor should be at least 1:5 (e. g. 50 µl sample + 200 µl diluent). A 1:100 dilution is useful for urine samples (e. g. 5 µl urine + 495 µl diluent). If samples generate values outside the standard curve, the dilution factor may be varied. Store the undiluted samples at –20 °C.

Assay procedure

It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, standard curve and samples as directed in the previous section.
2. Pipette 50 µl of samples, standards or diluent (as negative control) into the wells.
3. Immediately add 50 µl of HRP conjugate to each well.
4. Mix gently.
5. Seal wells with adhesive strip and incubate for 2 hours at room temperature.
6. Aspirate fluid from wells and wash three times with 300 µl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 20 minutes at room temperature in the dark.
9. Add 100 µl of stop solution to each well.
10. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

Calculation of results

Create a standard curve using computer software capable of generating a curve fit (four parameter fit; x-axis: log, albumin concentration; y-axis: linear, absorbance). As an alternative, draw a standard curve on semi-log paper (x-axis: log, albumin concentration; y-axis: linear, absorbance). The albumin concentrations can be calculated from the standard curve. The calculated concentrations must be multiplied by the sample dilution factor.

If the absorbance of some samples is outside the standard curve a subsequent determination with changed samples dilutions will provide a proper result (for example 1:20 instead of 1:5 for albumin concentrations above standard 7).