Albumin (Rat) ELISA

Catalog Number:
ALB21-K01 (1 x 96 wells)
ALB21-K04 (4 x 96 wells)
ALB21-K08 (8 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

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Introduction

The Eagle Biosciences Rat Albumin ELISA Assay Kit is designed for the quantitative determination of albumin in rat urine samples. The Rat Albumin ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

When blood is filtered in the glomeruli of kidneys, highly molecular substances are held back. Therefore, urine is nearly protein free. In case of kidney damages, small proteins like albumin occur 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. Rat albumin has been pre-coated onto a microplate. During incubation the binding of an enzyme-linked anti-albumin(rat)-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 Rat Albumin ELISA Assay Kit at 2-8 °C.
- The Rat Albumin ELISA Assay Kit is for research use only. Not for use in diagnostic procedures.
- Do not use the reagents beyond the expiration date marked on box label.
- Please read the instructions carefully before using the Rat Albumin ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Rat Albumin ELISA Assay 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 of the Rat Albumin ELISA Assay Kit 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 6	standard conc. Undiluted	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 urine samples with diluent. A 1:100 dilution is useful (e. g. 5 µl urine + 495 µl diluent). If samples generate values outside the standard curve, the dilution factor may be varied. To exclude matrix effects the dilution factor should be at least 1:40. If large quantities of diluted urine are secreted (for example from diabetic animals) the sample dilution may be reduced up to 1:10. Store urine samples at –20 °C.

Assay procedure

It is recommended that all samples and standards of the Rat Albumin ELISA Assay Kit be assayed in duplicate.

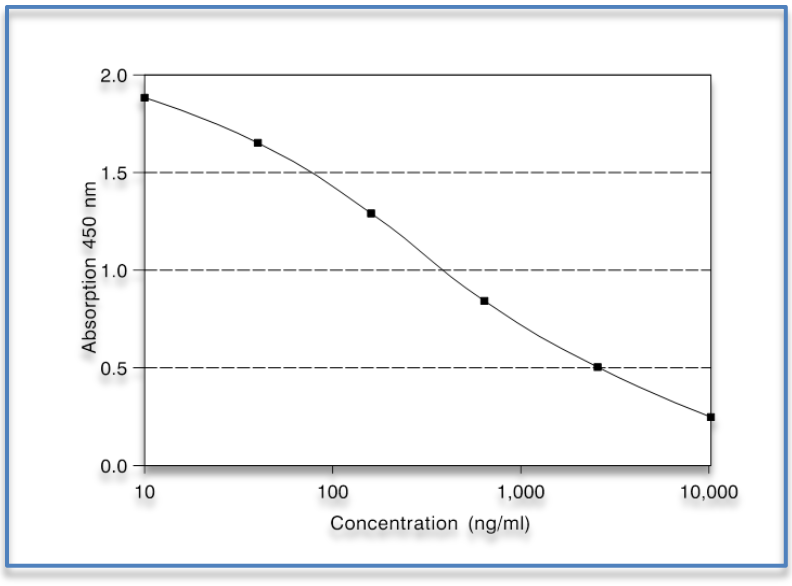
1. Prepare all reagents, standard curve and samples as directed in the previous section.
2. Pipette 50 µl of samples, standards, positive control or diluent (as negative control) into the wells.
3. Immediately add 50 µl of HRP conjugate to each well.
4. Mix gently.
5. Seal wells with adhesive strip and 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 10 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. 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 urine dilutions will provide a proper result (for example 1:2000 instead of 1:100 for albumin concentrations above standard 6).

Typical Standard Curve



Performance Characteristics

- *Standard curve:*
  6 standards between 10 ng/ml and 10240 ng/ml
- *Sensitivity:*
  0.4 µg/ml (sample dilution 1:40)
- *Sample material:* Urine
- *Intraassay precision (CV):*
  (sample dilution 1:100, n=12)
  at 8.5 µg/ml: 20.8%
  at 206 µg/ml: 10.2%
  at 1254 µg/ml: 12.9%
- *Cross Reactivity:* Albumin (bovine) <0,1%

Materials provided:

Number of determinations/Catalog No.	1x 96	4x 96	8x 96
Microplate strips, albumin (rat) coated	12x 8	4x (12x 8)	8x (12x 8)
Wash buffer, 10fold conc. ✦	40 ml	100 ml	2x 100 ml
Diluent, ready to use ✦	100 ml	2x 100 ml	4x 100 ml
Standard concentrate, 10240 ng/ml ✦	1 ml	2,5 ml	4 ml
Positive control, ready to use ✦	0.5 ml	1 ml	2 ml
Anti-albumin-Ab., HRP conjugate, 100fold	0.1 ml	0.3 ml	0.5 ml
TMB substrate, ready to use	12 ml	45 ml	2x 45 ml
Stop solution, ready to use (0.5 M sulphuric acid)	12 ml	45 ml	90 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 urine samples with diluent (recommended 1:100)
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 hour at room temperature
4. Wash three times with 300 µl of wash buffer
5. Dispense 100 µl of TMB substrate solution
6. Incubate for 10 minutes at room temperature in the dark
7. Add 100 µl of stop solution
8. Measure absorption at 450 nm

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.