Catalog #80662

V.4

Rat Albumin ELISA Kit Instructions

For the quantitative determination of albumin in rat serum, plasma, and urine

Catalog #80662
96 Assays

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

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A. Intended Use

The Rat Albumin ELISA kit is for the quantitative determination of albumin in rat serum, plasma, and urine. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Albumin is synthesized in the liver, and serum albumin constitutes approximately 55-60% of total serum proteins. Albumin contributes to homeostasis through mechanisms of hemodynamics, transport and nutrition. Albumin is found both intra and extravascularly in all mammals and many lower vertebrates.

C. Principle of the Assay

The Rat Albumin ELISA kit is a sandwich ELISA. An unknown amount of albumin present in the sample binds with anti-albumin antibodies bound to the microplate. After washing to remove unbound proteins, HRP-conjugated anti-albumin antibodies are added and form a complex with the albumin complex present in the wells. TMB substrate is then added to measure the concentration of albumin present.

D. Kit Storage

1. Upon receipt of the Rat Albumin ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD	Standard (Lyophilized)	1 vial
DIL	Diluent (5X Concentrate)	1 x 50 mL
AB CONJ	Antibody Conjugate (100X Concentrate)	1 vial/150 µL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled or deionized water
Polypropylene microtubes
Volumetric flasks
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (2 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Plasma: Collect blood into a tube containing an anticoagulant such as heparin (final concentration: 1 unit/mL), EDTA (final concentration: 0.1%), or sodium citrate (final concentration: 0.76%), and centrifuge for 20 min at 2,000 x g.

Serum: Collect blood, allow to clot, and centrifuge for 20 min at 2,000 x g.

Urine: Collect urine and store.

The samples should be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples with excessive hemolysis should not be used.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standard
   The standard is provided in lyophilized form. Dilute the standard with 1 mL of distilled or deionized water. Mix gently until dissolved. After reconstitution, the standard concentration will be 101.4 µg/mL. The standard should be stored frozen for future use and appropriately aliquoted in appropriate volumes prior to being frozen. Working standards should be prepared immediately prior to use as described in Section I.2 and are stable for up to 8 hours. The working standard concentrations are 0, 6.25, 12.5, 25, 50, 100, 200, and 400 ng/mL.
3. Diluent (5X Concentrated)
   The diluent has to be diluted 1:5 with distilled or deionized water prior to use. For example, 50 mL of diluent must be diluted with 200 mL of distilled or deionized water. Diluent is stable for at least one week after dilution.

4. Antibody Conjugate (100X Concentrated)
   The antibody conjugate has to be diluted 1:100 with 1X Diluent prior to use. For each test strip, mix 10 µL of antibody conjugate with 990 µL of 1X Diluent. Mix uniformly, but gently. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark. Accordingly, working conjugate solution should be prepared only as needed just prior to use.
5. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals. Wash buffer is stable for at least one week after dilution.
6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.

I.2. Preparation of working standards

1. Pipette 505 µL of 1X diluent and 2 µL of the reconstituted standard (101.4 µg/mL) into a polypropylene microtube labeled 400 ng/mL, and mix thoroughly.
2. Dispense 250 µL of 1X diluent into six polypropylene microtubes labeled 200, 100, 50, 25, 12.5, and 6.25 ng/mL,
3. Dispense 250 µL of the 400 ng/mL standard into the 200 ng/mL microtube, and mix thoroughly.
4. Dispense 250 µL of the 200 ng/mL standard into the 100 ng/mL microtube, and mix thoroughly.
5. Dispense 250 µL of the 100 ng/mL standard into the 50 ng/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 500 µL of 1X diluent into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 400, 200, 100, 50, 25, 12.5, 6.25, and 0 ng/mL.

Please note: Working standards should be prepared immediately prior to use and are stable for up to 8 hours.

I.3. Dilution of samples

Samples and need to be diluted with 1X diluent for use with the assay.

Serum/Plasma Samples: A sample dilution of 1:1,000,000 using 2 µL of sample is generally suitable. To prepare the 1:1,000,000 dilution, mix 2 µL of sample with 1,998 µL of 1X Diluent (1:1,000). Then add 2 µL of the 1:1,000 dilution to 1,998 µL of 1X Diluent. You now have 1:1,000,000 dilution.

Urine Samples: A sample dilution of 1:500 using 2 µL of sample is generally suitable. To prepare the 1:500 dilution, mix 2 µL of sample with 998 µL of 1X Diluent. You now have 1:500 dilution.

Since rat albumin levels can vary, dilution ratios may need to be adjusted as appropriate.

I.4. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or working standard.
2. Incubate plate for 30 min at room temperature. Keep plate covered and level.
3. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. In each well, add 100 µL of diluted Antibody Conjugate and mix well by repeated pipetting.
5. Incubate plate for 30 min at room temperature. Keep plate covered and level.
6. Aspirate well contents and wash four times using 300 µL of 1X Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of the Substrate Solution in each well.
8. Incubate plate for 10 mins in dark room at room temperature.
9. Stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.5. Determining the albumin concentration

1. Using computer software, construct the albumin calibration curve by plotting the mean absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding albumin concentration on the X axis. A four parametric logistic (4-PL) curve fit or second order polynomial (quadratic) are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Rat albumin concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor to obtain the final albumin concentration (expressed in ng/mL).
   Note: *Samples with high rat albumin concentrations (ie. fall above the range of the assay) should be further diluted and rerun.*

J. Performance characteristics

J.1. Assay range

The Rat Albumin ELISA Kit has an assay range from 6.25 – 400 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

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