

RAT ALBUMIN

Immunoperoxidase Assay for Determination of ALBUMIN in Rat Samples

DIRECTIONS FOR USE

Version L5.0

INTENDED USE

The ALBUMIN test kits are a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring ALBUMIN in serum, plasma or urine of rats.

INTRODUCTION

Albumin (Alb) is an amazing polyfunctional protein contributing to homeostasis through mechanisms of hemodynamics, transport and nutrition. Albumin is found both intra and extracellularly in all mammals and many lower vertebrates. It is a molecule of about 67,000 daltons, synthesized by the liver. Normally only very trace amounts of albumin escape reabsorption by kidney glomeruli and is excreted into the urine. Many occult diseases can cause kidney damage which may result in excessive amounts of serum proteins, including albumin, to be excreted by the kidney and into the urine. This ELISA kit can be used to measure albumin in serum, tissue extracts and other biological fluids.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the albumin present in serum sample reacts with the anti-Alb. antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound serum proteins by washing, anti-Alb. antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound serum Alb. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of Albumin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Albumin in the test sample. The quantity of Albumin in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for serum dilution.

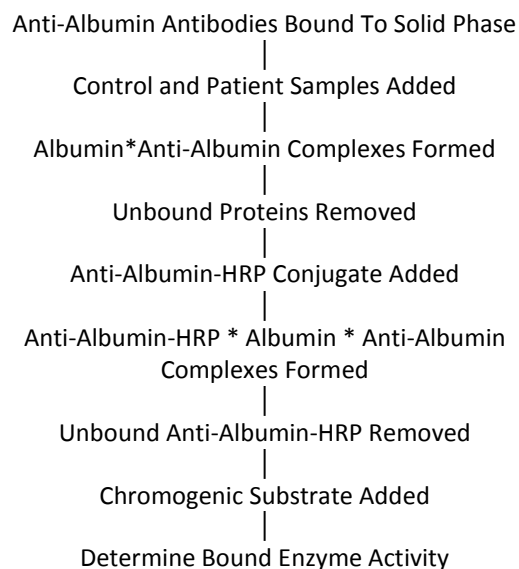


Figure 1.

REAGENTS

(Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE

One bottle containing 50 ml of a 5X concentrated phosphate buffered saline (PBS) solution containing bovine serum albumin, 0.25% Tween, and 0.25% Proclin 300 as a preservative.

2. WASH SOLUTION CONCENTRATE

One bottle containing 50 ml of a 20X concentrated phosphate buffered saline (PBS) solution containing 1% Tween.

3. ENZYME-ANTIBODY CONJUGATE 100X

One vial containing 200 μ L of affinity purified anti-Rat ALBUMIN antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN-SUBSTRATE SOLUTION

One vial containing 12 mL of 3,3',5,5'-

tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION

One vial containing 12 ml 0.3 M sulfuric acid.

WARNING: Avoid contact with skin.

6. ANTI-RAT ALBUMIN ELISA MICRO PLATE

Eight removable twelve (12) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Rat Albumin.

7. RAT ALBUMIN CALIBRATOR

One vial containing a lyophilized Rat Albumin calibrator.

FOR IN VITRO USE ONLY

REAGENT PREPARATION

1. DILUENT CONCENTRATE

The Wash Solution supplied is a 5X Concentrate and must be diluted 1:5 with distilled or deionized water.

2. WASH SOLUTION CONCENTRATE

The Wash Solution supplied is a 20X Concentrate and must be diluted 1:20 with 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.

3. ENZYME-ANTIBODY CONJUGATE 100X

The required amount of working conjugate solution for the entire microtitre plate is prepared by adding 100 µL Enzyme-Antibody Conjugate to 10 mL of Diluent. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN-SUBSTRATE SOLUTION

Ready to use as supplied.

5. STOP SOLUTION

Ready to use as supplied.

6. ANTI-RAT ALBUMIN ELISA MICRO PLATE

Ready to use as supplied.

7. RAT ALBUMIN STANDARDS

Add 1.0 ml of distilled or de-ionized water to the Rat Albumin Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 121 µg/ml (**the reconstituted calibrator should be aliquoted and frozen if future use is intended**). Rat Albumin standards need to be prepared immediately prior to use (see the following

chart). Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to 1x Diluent	Volume of 1x Diluent
1	400	2 µl Rat Albumin Calibrator	603 µl
2	200	300 µl standard 1	300 µl
3	100	300 µl standard 2	300 µl
4	50	300 µl standard 3	300 µl
5	25	300 µl standard 4	300 µl
6	12.5	300 µl standard 5	300 µl
7	6.25	300 µl standard 6	300 µl

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. DILUENT

The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. WASH SOLUTION

The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. ENZYME-ANTIBODY CONJUGATE

Undiluted horseradish peroxidase anti-ALB conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for one day.

4. CHROMOGEN-SUBSTRATE SOLUTION

The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

5. STOP SOLUTION

The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

6. ANTI-RAT ALBUMIN ELISA MICRO PLATE

Anti-Rat ALB coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. RAT ALBUMIN STANDARDS

The lyophilized Rat Albumin Calibrator should be stored

at 4C or frozen until reconstituted. The reconstituted calibrator should be aliquoted and stored frozen (avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use and are stable for 1 day.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the standard solutions should be within 20 % of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. Specimens may be shipped at room temperature and then stored refrigerated at 2-8°C if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

3. Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

MATERIAL PROVIDED

See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µL to 200µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions

- Timer
- Vortex mixer

ASSAY PROTOCOL

DILUTION OF YOUR SAMPLES

The assay for quantification of albumin in serum requires that each test sample be diluted before use. A 1:500 dilution is appropriate for most urine samples while serum or plasma samples may need to be diluted 1:1,000,000. **For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required.**

1. To prepare a 1:500 dilution of sample, transfer 2 µL of sample to 998µL of diluent. This gives you a 1:500 dilution.
2. To prepare a 1:1,000,000 transfer 1.0 µl of serum or plasma to 999 µl of diluent. You now have a 1:1,000 dilution. Next mix 1.0 µl of your 1:1,000 dilution with 999 µl of diluent. You now have a 1:1,000,000 dilution.

PROCEDURE

Bring all reagents to room temperature before use.

1. Add 100 µL of Diluent to each of the wells in A1 & A2. These will serve for an evaluation of the background associated with the assay.
2. Pipette 100 µL of
Standard 1 (400 ng/ml) into wells A3 & A4
Standard 2 (200 ng/ml) into wells A5 & A6
Standard 3 (100 ng/ml) into wells A7 & A8
Standard 4 (50 ng/ml) into wells A9 & A10
Standard 5 (25 ng/ml) into wells A11 & A12
Standard 6 (12.5 ng/ml) into wells B1 & B2
Standard 7 (6.25 ng/ml) into wells B3 & B4

3. Pipette 100 µL of serum sample (test sample 1) into wells B5 & B6. The next sample goes in wells B7 & B8, the next in B9 & B10 and so on.

4. Incubate the micro titer plate at 22°C (room temperature) for thirty (30 ± 2) minutes. Keep plate level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually; completely fill wells with wash buffer, invert the plate and vigorously pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 μ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (room temperature) for thirty (30 ± 2) minutes.

8. Wash and blot the wells as described in Step 6.

9. Pipette 100 μ L of TMB Substrate Solution into each well.

10. Incubate at room temperature for precisely ten (10) minutes.

11. After ten (10) minutes, add 100 μ L of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample. Value should not be greater than 0.2.

2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the Albumin concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the Assays for specific ALB require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, washing thoroughly and accuracy of reagent and sample pipettings.