

[Rat Urinary Albumin Assay Kit (S-type)]

(Code No.:AKRAL-020S)

Please, read this instruction carefully before use.

This kit is manufactured by Shibayagi Co., Ltd.

Use only the current version of Instruction Manual enclosed with the kit!

1. Intended use

Rat Urinary Albumin Assay Kit is a research reagent for an autoanalyzer to measure albumin in rat urine or serum using anti-rat albumin antibody. This is intended for research use only.

2. Storage and expiration

When the complete kit is stored at 2-8°C, the kit is stable until the expiration date shown on the label on the box. Opened reagents should be used as soon as possible to avoid loss in optimal assay performance caused by storage environment.

3. Introduction

Albumin is mostly a simple hydrophilic protein present in cells and body fluids. Albumin is synthesized in the liver, and serum albumin (69 kDa, pI 4.9) occupies 56-60% of total serum proteins. Because of its large population, albumin is very important in maintaining plasma osmotic pressure. Albumin can bind hydrophobic physiological substances e.g. fatty acids, bilirubin, and thyroxine and contributes the transfer of these substances. Concentration of albumin in serum is lowered in liver cirrhosis, malnutrition, and pyrexial diseases due to decreased biosynthesis or increased consumption of albumin in, and also by secretion into urine in renal damage. In normal human subjects, excretion of albumin into urine is very little, about 30mg/day, but increased in glomerulonephritis, nephritic syndrome, and diabetic nephropathy. Urinary albumin sometimes increases in pyrexia, hypertension, congestive heart failure (CHF), and urinary tract infection (UTI). Even in healthy human, a transient upraise of urinary albumin is observed after hard exercise, muscle work, bathing in high temperature water, mental excitement, stress, intake of much protein, and before menstruation. These are called physiological or functional or sports proteinuria (albuminuria). Orthostatic proteinuria (albuminuria) is observed in teenagers. Rare hereditary analbuminemia in human is really albumin deficiency. A model analbuminemia rat has been established by Dr. Sumi Nagase which has been derived from Sprague-Dawley strain, and is called Nagase analbuminemia rat (NAR). Routine measurement of serum albumin is conveniently made with Shibayagi's TIA assay kit for automatic analyzers. Shibayagi's Albumin ELISA Kit can measure urinary albumin with high sensitivity, and is also applied to *in vitro* albumin biosynthesis system, checking of contamination with albumin in biological active substance preparations obtained by culture system, and liver transplantation experiments using NAR.

4. Assay principle

In Shibayagi's Rat Urinary Albumin Assay Kit, albumin in samples and antibody of Reagent 2 bring about antigen-antibody reaction, and it makes turbidity. Since this turbidity depends on its antigen concentration, it makes possible to detect albumin concentration by measuring this turbidity at 340 nm/700nm.

5. Precautions

- Beginners are advised to use this kit under the guidance of experienced person for autoanalyzer.
- Do not use this product for other purpose or in other way except stated in this manual.
- Note that operation of autoanalyzers is different in each machine. Follow your own machine's protocol.
- Do not drink, eat or smoke in the areas where assays are carried out.
- This kit contains components of animal origin. These materials should be handled as potentially infectious.
- Be careful not to allow the reagent solutions of the kit to touch the skin, eyes and mucus

membranes. In case of contact with these wash skin/eyes thoroughly with water and seek medical attention, when necessary.

- Wear gloves and eye and clothing protection when handling these reagents.
- The materials must not be pipetted by mouth.
- Use clean laboratory glassware.
- Do not use with different lot numbers. Performance or character is different in each lot number. Also avoid extension of reagents within same lot as it may influence assay results.
- Store all reagents in a refrigerator at 2-8°C strictly. Do not freeze reagents.

6. Technical tips

- Be careful to avoid any contamination of assay samples and reagents. We recommend using disposal pipette tips.
- Be careful not to make bubbles in samples and every reagent. Check if there are bubbles before setting into autoanalyzer. If so, remove them using filter paper before starting assay.
- Calibrate the analyzer before assay.
- Used samples and consumables should be rinsed in 1% formalin, 2% glutal aldehyde, or more than 0.1% sodium hypochlorite solution for more than 1 hour, or be treated by an autoclave before disposal. Also, dispose consumable materials and unused contents in accordance with applicable regional/national regulatory requirements.

7. Reagents supplied

Components	State	Amount
(1) Reagent 1 (Buffer)	Ready for use.	18.5 ml x 1
(2) Reagent 2 (Anti-rat albumin solution)	Ready for use.	6.6 ml x 1
(3) Standard rat albumin* (derived from rat)	Concentrated. Use after dilution.	2.0 ml x 1
(4) Diluent solution for standard albumin	Ready for use.	4.0 ml x 1
Instruction Manual/Parameter table (Hitachi 7070, 7170, 7080, 7180)	—	1 copy

*Concentration of standard rat albumin is indicated on its bottle label.

8. Equipments or supplies required but not supplied Use as a check box

- An autoanalyzer
- Purified water (distilled water)
- Physiological saline
- Test tubes for preparation of standard solution series
- Pipettes (disposable tip type)
- A vortex-type mixer.

9. Preparation of reagents

- ◆ Prepare reagent solutions in appropriate volume for your assay. Do not store the diluted reagents.
- ◆ Do not use the reagents after expiration date indicated on the label of the box.

● Reagents ready for use

[Reagent 1 (Buffer)]

Storage and stability

Store at 2-8 °C. It is stable until expiration date.

[Reagent 2 (Anti-rat albumin solution)]

Storage and stability

Store at 2-8 °C. It is stable until expiration date.

[Diluent solution for standard albumin]

Storage and stability

Store at 2-8 °C. It is stable until expiration date.

● **Concentrated reagents**

[Standard rat albumin]

Dilute standard rat albumin serially 3x using diluent solution for standard albumin.

To make 6 points of standard curve specimen, use diluent solution for standard albumin for the 0 µg/ml.

The example is shown below.

Concentration (µg/ml)	500	167	55.6	18.5	6.17	0
Standard solution (µl)	orig.sol.: 500	250*	250*	250*	250*	0
Buffer solution (µl)	0	500	500	500	500	500

*One rank higher standard.

Storage and stability

Store the unused reagents at 2-8 °C. It maintains stability until expiration date.

Diluted standard solutions can be stable within a week if stored 2-8 °C.

10. Preparation of samples

This kit is intended to measure albumin in rat urine or serum.

Urinary samples

Run your assay soon after collection of urinary samples. If you would like to store urinary samples for a long time, store them below -35 °C. Turbid samples or those containing insoluble materials should be centrifuged or filtered before testing to remove any particulate matter.

Serum samples

Serum samples should be immediately assayed after collection or stored below -35 °C for a long-time storage. Dilute samples to the proper rate for assay. [Hemolytic and hyperlipemic serum samples are not suitable.](#)

*If presence of interfering substance is suspected, examine by dilution test at more than 2 points.

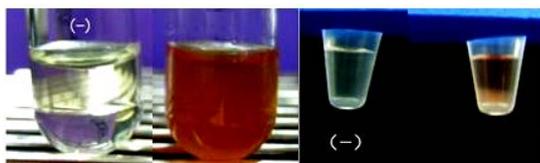
*Dilute samples with purified water for an autoanalyzer, or dilution of samples should be made in a test tube using saline solution. Obtained value should be raised its dilution rate to be assay value.

*Before starting assay, shake thawed samples sufficiently. Do not repeat freeze-and-thaw cycles.

*Adjust samples' pH within 6.5-7.5.

*** To avoid influence of blood (high lipid or hemolysis, etc.), if your original samples have heavy chyle or hemolysis as the pictures below, do not use them for assay. Abnormal value might be obtained with hemolysis above 200mg/dL with this kit.**

Normal Hemolysis Normal Hemolysis



Color sample for hemolytic sample: 200mg/dL

Normal Chyle Normal Chyle

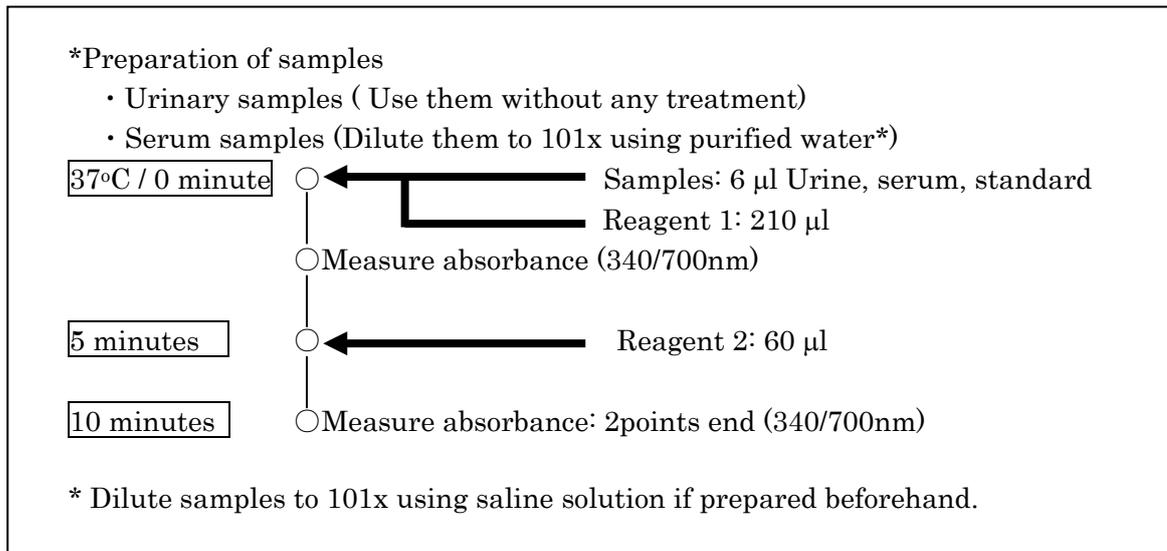


Color sample for chylous sample: 1,000FTU

11. Assay procedure

For Hitachi 7180 autoanalyzer

*Note that operation of autoanalyzer is different depending on the type of machines



12. Performance characteristics

● Assay range

The assay range of the kit is 6.17 ~ 500 µg/ml.

Regarding prozone or setting of panic value, please contact: syc-info@shibayagi.co.jp

● Precision of assay

Within assay variation (3 samples, 5 replicates assay), Mean CV is less than 5%

● Reproducibility

Between assay variation (3 samples, 4 days, 3 replicates assay), Mean CV is less than 5%

● Dilution linearity test

Sample was serially diluted by 4 steps and measured.

The dilution curve showed linearity with R = 0.999.

13. Reference data

Strain	Week old	Gender	Number	Sample	Mean	Standard deviation
CD	6w	Male	5	Urine	19.4 µg/ml	0.13 µg/ml

*Urine was not diluted.

* These data should be considered as guidance only. Each laboratory should establish its own normal and pathological reference ranges for albumin levels independently.

[Storage condition] Store the kit at 2-8°C (Do not freeze).

[Term of validity] Six months from production (Expiration date is indicated on the container.)

Shibayagi

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