Chondrex, Inc.

Rat Albumin Detection Kit

Catalog # 3020

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Albumin is the most abundant protein in plasma, accounting for more than half of the measured serum protein. It consists of a single polypeptide chain of 585 amino acids with a molecular weight of 66.5 kDa. Because of its high molecular weight and concentration in plasma, albumin is critical in maintaining plasma osmotic pressure.

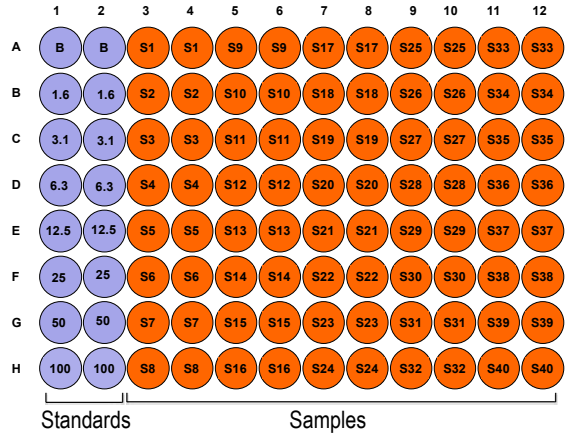
In human, plasma albumin levels are significantly decreased in patients with severe liver disease (2) or poor nutritional status (5), since albumin synthesis takes place exclusively in the liver (1). Alternatively, low plasma albumin levels occur due to increased capillary leakage caused by critical illnesses such as kidney disease (3), inflammation (4), sepsis, and major surgical stress (6). In addition, excretion of albumin into urine (albuminuria) is very low in healthy individuals, but it is significantly increased by renal damage in glomerulonephritis (7) and diabetic nephropathy (8,9).

In fact, rat disease models of sepsis (10), glomerulonephritis (11) and diabetic nephropathy (12) demonstrate comparable albuminuria with human patients, suggesting that rat urinary albumin levels are useful for evaluating disease severity and treatment efficacy. Chondrex, Inc. provides a Rat Albumin Detection Kit (Catalog # 3020) and a Rat Urinary Protein Assay Kit (Catalog # 9040) depending on the purpose of the study.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Rat Albumin Standard	1 vial	100 ng, lyophilized	-20°C
Capture Antibody	1 vial	50 µl, 1 mg/ml	-20°C
Detection Antibody	1 vial	50 µl	-20°C
Solution A - Capture Antibody Dilution Buffer	1 bottle	10 ml	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer	1 bottle	50 ml	-20°C
OPD	2 vials	Lyophilized	-20°C
Chromogen Dilution Buffer	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 ml	-20°C
Wash Buffer, 20X	1 bottle	50 ml	-20°C
96-Well ELISA Plate	1 each	8-well strips x 12	-20°C

Figure 1 - A Standard Assay Layout



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NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate (Figure 1).

Note 2: Warm up all buffers to room temperature before use.

Note 3: Partially used reagents may be kept at –20°C.

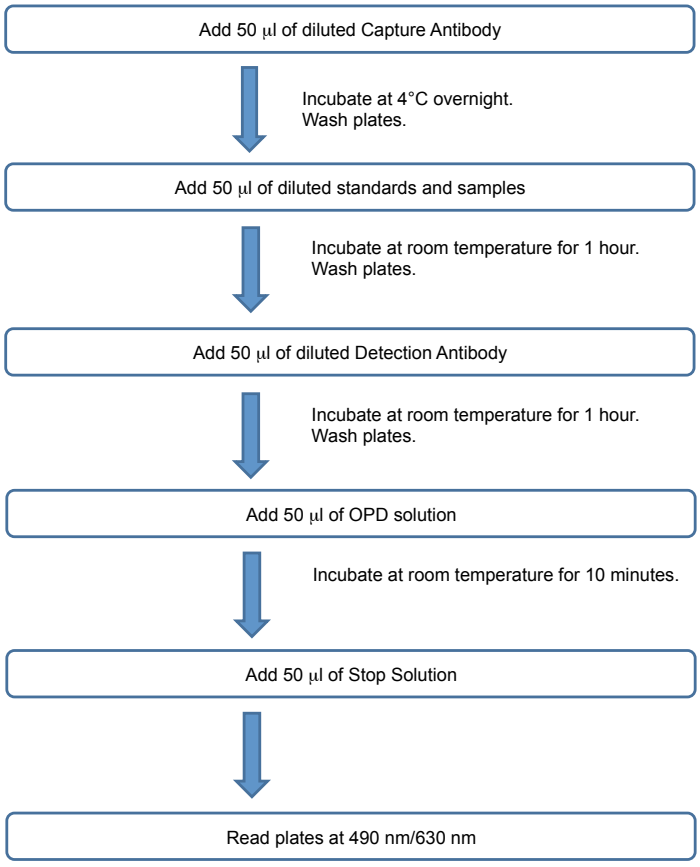
Note 4: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipette, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

Note 7: This kit contains components from non-infectious animals, but should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE



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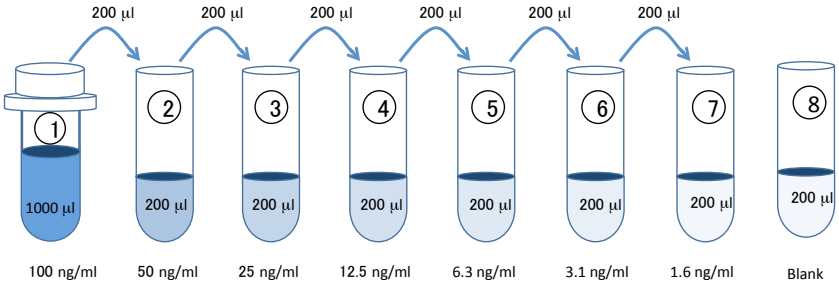
1. **Coat Plate with Capture Antibody**: Centrifuge a Capture Antibody vial at 3000 rpm x 1 minutes. Dilute one vial of Capture Antibody with 5 ml of Capture Antibody Dilution Buffer (Solution A). Add 50 µl of capture antibody solution to each well and incubate at 4°C overnight.

2. **Dilute Wash Buffer**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

3. **Prepare Standard Dilutions**: The recommended standard range is 1.6 -100 ng/ml. Dissolve one vial of rat albumin standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 100 ng/ml standard. Then serially dilute it with Solution B. For example, mix 200 µl of the standard (100 ng/ml) with an equal volume of Solution B to make a 50 ng/ml solution, and then repeat it five more times for 25, 12.5, 6.3, 3.1, and 1.6 ng/ml solutions. The remaining 100 ng/ml standard stock may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



4. **Prepare Sample Dilutions**: Centrifuge the samples at 10,000 rpm for 5 minutes to remove insoluble materials in urine or serum samples. Dilute samples 1:500-1:2,000,000 with Solution B depending on the estimated albumin levels in the samples. It is recommended to use 2-3 different dilutions if the sample albumin level is unknown.

   Note: Due to the high dilution of samples, 1% BSA/PBS pH 7.4 can be used for sample dilution. However, the final diluted samples for ELISA should include at least 50% of Solution B. For example, if your sample is diluted at 1: 20,000; take 10 µl of sample and add to 990 µl of 1% BSA/PBS pH 7.4 (1:100). Then repeat this dilution for a 1:10,000 dilution. Mix 100 µl of the diluted sample (1:10,000) with an equal volume of Solution B for a 1:20,000 dilution.

5. **Add Standards and Samples**: Use the plate layout as shown in Figure 1. Add 50 µl of Solution B (blank), standards, and samples to designated wells in duplicate. Incubate at room temperature for 1 hour.

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Detection Antibody**: Centrifuge a Detection Antibody vial at 3000 rpm x 1 minutes. Dilute one vial of Detection Antibody with 5 ml of Standard/Sample/Detection Antibody Dilution Buffer (Solution B). Add 50 µl of detection antibody solution to each well and incubate at room temperature for 1 hour. The remaining dissolved detection antibody solution can be stored at -20°C for use in a second assay.

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **OPD**: Dissolve one vial of OPD in 10 ml of Chromogen Dilution Buffer. Add 50 µl of OPD solution to each well immediately after washing the plate. Incubate for 10 minutes at room temperature.

10. **Stop**: Stop the reaction with 50 µl of 2N sulfuric acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 490 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

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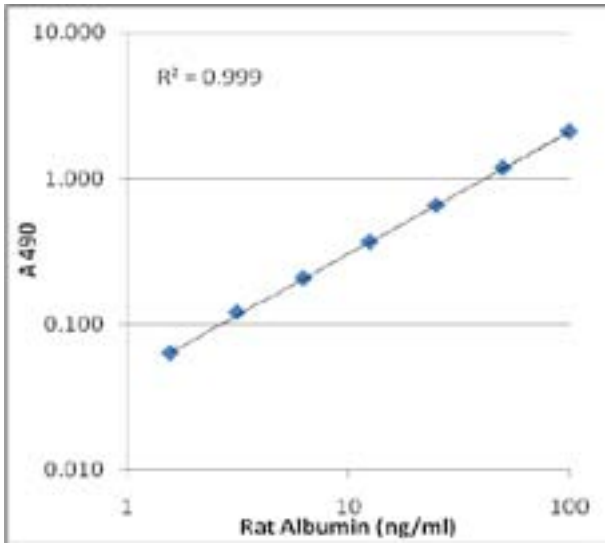
CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank (B), standards, and test samples.
2. Subtract the “blank” (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of rat albumin (ng/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is from 1.6-100 ng/ml.
4. The ng/ml of rat albumin in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for rat albumin assay



PRECISION TEST RESULTS

Table 1 - Precision of Rat Albumin Detection Kit (catalog # 3020)

Test	2.7 ng/ml	12 ng/ml	48 ng/ml
Inter-Assay CV (%)	4.1	8.7	7.1
Intra-Assay CV (%)	5.6	6.5	2.1
Spike Test*	93.2	93.9	90.3

*Known amounts of rat albumin were added to normal rat serum samples diluted with Sample/Standard/Detection Antibody Dilution Buffer.

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REFERENCES

1. Oettl K, Stadlbauer V, Petter F, et al. Oxidative damage of albumin in advanced liver disease. Biochim Biophys Acta. 2008;1782(7-8):469-473.
2. Don BR, Kaysen G. Serum albumin: relationship to inflammation and nutrition. Semin Dial. 2004;17(6):432-437.
3. MILLER LL, BLY CG, WATSON ML, BALE WF. The dominant role of the liver in plasma protein synthesis; a direct study of the isolated perfused rat liver with the aid of lysine-epsilon-C14. J Exp Med. 1951;94(5):431-453.
4. Eustace JA, Astor B, Muntner PM, Ikizler TA, Coresh J. Prevalence of acidosis and inflammation and their association with low serum albumin in chronic kidney disease. Kidney Int. 2004;65(3):1031-1040.
5. Moshage HJ, Janssen JA, Franssen JH, Hafkenscheid JC, Yap SH. Study of the molecular mechanism of decreased liver synthesis of albumin in inflammation. J Clin Invest. 1987;79(6):1635-1641.
6. Fleck A, Raines G, Hawker F, et al. Increased vascular permeability: a major cause of hypoalbuminemia in disease and injury. Lancet. 1985;1(8432):781-784.
7. Li Y, Wang J, Zhu X, Feng Q, Li X, Feng X. Urinary protein markers predict the severity of renal histological lesions in children with mesangial proliferative glomerulonephritis. BMC Nephrol. 2012;13:29.
8. Roshan B, Stanton RC. A story of microalbuminuria and diabetic nephropathy. J Nephropathol. 2013;2(4):234-240.
9. Newman DJ, Mattock MB, Dawnay AB, et al. Systematic review on urine albumin testing for early detection of diabetic complications. Health Technol Assess. 2005;9(30):iii-vi, xiii-163.
10. Adembri C, Sgambati E, Vitali L, et al. Sepsis induces albuminuria and alterations in the glomerular filtration barrier: a morphofunctional study in the rat. Crit Care. 2011;15(6):R277.
11. Zhang S, Xin H, Li Y, et al. Skimmin, a Coumarin from Hydrangea paniculata, Slows down the Progression of Membranous Glomerulonephritis by Anti-Inflammatory Effects and Inhibiting Immune Complex Deposition. Evid Based Complement Alternat Med. 2013;2013:819296.
12. Ma R, Liu L, Liu X, Wang Y, Jiang W, Xu L. Triptolide markedly attenuates albuminuria and podocyte injury in an animal model of diabetic nephropathy. Exp Ther Med. 2013;6(3):649-656.

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