Mouse Urinary Albumin Detection Kit

Catalog # 3012

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INTRODUCTION

Proteinurea is a common symptom of nephritis in humans and experimental animals, and is an important marker for evaluating disease severity regardless of the type of nephritis. Moreover, proteinurea is determined as the total amount of protein (serum proteins such as globulins and albumin) excreted into a 16-hour urine collection, which is distinctly more accurate than determining the urinary protein concentration, due to large variations in urine volume between individual animals. The turbidity method is a commonly used urinary protein assay method, because it is accurate, easy, and economical.

However, mouse kidneys leak serum components such as bilirubin, resulting in overestimated urinary protein levels, thus proteinuria is not a suitable marker for the evaluation of nephritis severity. On the other hand, albumin, a serum protein with a relatively small molecular weight, and typically the first protein observed in the urine when kidney dysfunction begins to develop, is a more suitable marker to evaluate the severity of nephritis in mouse models.

Chondrex's Mouse Urinary Albumin Detection Kit (catalog # 3012) is designed to specifically determine mouse albumin in 40 urine samples within 4 hours using a sandwich immunoassay method. In mouse nephritis models, such as immune complex-induced glomerulonephritis (ICGN), a mouse is deemed nephritic if the total albumin content in a 16-hour urine collection is greater than 1 mg. It is important to note that urinary albumin levels can reach up to 50-200 mg in a 16-hour urine collection, depending on the severity of nephritis.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Mouse Albumin Standard	1 vial	100 ng, lyophilized	-20°C
Capture Antibody (Goat Anti-Mouse Albumin Polyclonal Antibody)	1 vial	50 µl, 1 mg/ml	-20°C
Detection Antibody (Biotinylated Goat Anti-Mouse Albumin Polyclonal Antibody)	1 vial	Lyophilized	-20°C
Solution A - Capture Antibody Dilution Buffer	1 bottle	10 ml	-20°C
Solution B - Sample/Standard and Detection Antibody Dilution Buffer	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer	1 bottle	20 ml	-20°C
Streptavidin Peroxidase	2 vials	50 µl/vial	-20°C
OPD	2 vials	Lyophilized	-20°C
Chromagen 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

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NOTES BEFORE USING ASSAY

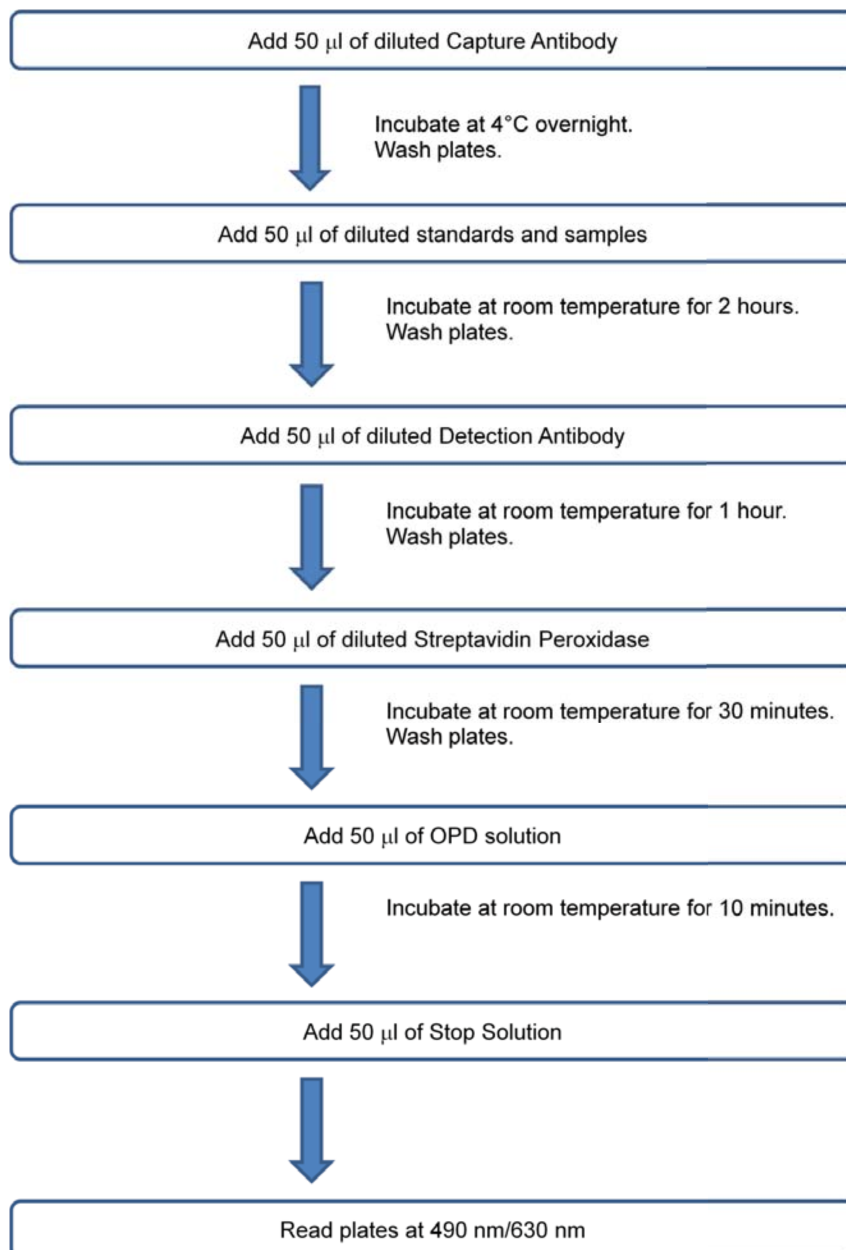
Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Partially used reagents may be kept at –20°C.

Note 3: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, place the bottle in warm water until crystals have dissolved completely.

Note 4: Measure exact volume of buffers using a serological pipette prior to diluting. Extra buffer is provided.

ASSAY PROCEDURE



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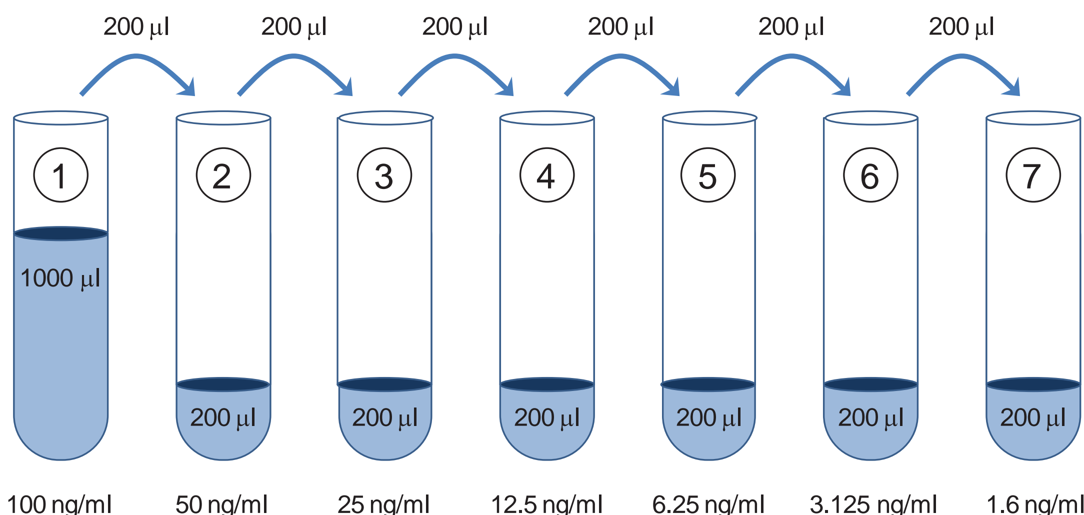
1. **Coat Plate with Capture Antibody**: Dilute one vial of Capture Antibody with 5 ml of Capture Antibody Dilution Buffer (Solution A). Add 50 µl of capture antibody solution to each well and incubate at 4°C overnight.

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 mouse albumin standard in 1 ml of Sample/Standard 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, 6.25, 3.125, 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 samples. Dilute samples 1:500-1:100,000 with Solution B depending on the estimated albumin content in the samples. It is recommended to use 2-3 different dilutions if the sample albumin level is unknown.

5. **Add Standards and Samples**: Add 50 µl of Solution B (blank), standards, and samples to designated wells in duplicate. Incubate at room temperature for 2 hours.

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**: Dissolve one vial of Detection Antibody in 5 ml of 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. **Add Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 50 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

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10. **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.*

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

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

13. **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.

CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank, 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 mouse albumin (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is from 1.6-100 ng/ml.

4. The ng/ml of mouse albumin in test samples can be calculated using regression analysis.

Figure 1 - A typical standard curve for mouse albumin assay

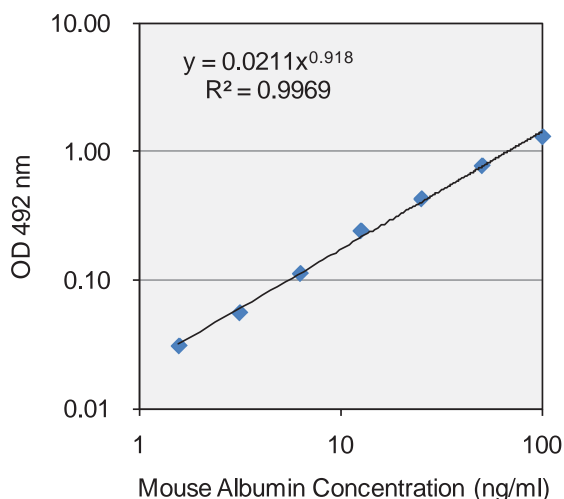

Table 1 - Reproducibility of data assayed by Mouse Urinary Albumin Detection Kit (catalog #3012)

Test	1.6 ng/ml	12.5 ng/ml	50 ng/ml
Inter-Assay CV (%)	2.1	2.6	1.7
Intra-Assay CV (%)	1.9	1.6	1.8
Spike Test*	98%	89%	96%

* Known amounts of mouse albumin was added to normal mouse urine pool, and then diluted with Sample/Standard Dilution Buffer B.

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