Mouse Albumin Detection ELISA Kit

Catalog # 3012

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify mouse albumin
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	100 - 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Urine and Serum
RECOMMENDED SAMPLE DILUTIONS:	1:500 (at least)
CHROMOGEN:	OPD (read at 490 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (1.6-1.9%)/Inter-Assay (1.7-2.6%)/Spiking Test (89-98%)
NOTES:	N/A

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INTRODUCTION

Proteinuria 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, proteinuria is determined as the total amount of proteins (serum proteins such as globulins and albumin) excreted into a 16-hour period, 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, Inc.'s Mouse Urinary Albumin Detection ELISA Kit (Cat # 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 (30121)	1 vial	100 ng, lyophilized	-20°C
Capture Antibody (30122) (Goat Anti-Mouse Albumin Polyclonal Antibody)	1 vial	50 µl, 1 mg/ml	-20°C
Detection Antibody (30123) (Biotinylated Goat Anti-Mouse Albumin Polyclonal Antibody)	1 vial	Lyophilized	-20°C
Solution A - Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (30106)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl/vial	-20°C
OPD (90021)	2 vials	Lyophilized	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
96-Well ELISA Plate	1 each	8-well strips x 12	-20°C

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ASSAY OUTLINE

Add 50 µl of diluted Capture Antibody Solution

↓ Incubate at 4°C overnight. Wash plates.

Add 50 µl of diluted Standards and Samples

↓ Incubate at room temperature for 2 hours. Wash plates.

Add 50 µl of diluted Detection Antibody Solution

↓ Incubate at room temperature for 1 hour. Wash plates.

Add 50 µl of diluted Streptavidin Peroxidase Solution

↓ Incubate at room temperature for 30 minutes. Wash plates.

Add 50 µl of diluted OPD Solution

↓ Incubate at room temperature for 10 minutes.

Add 50 µl of diluted Stop Solution

↓

Read plates at 490 nm/630 nm

PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	50	50	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	25	25	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	12.5	12.5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	6.3	6.3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	3.1	3.1	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	1.6	1.6	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards | Samples

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NOTES BEFORE USING ASSAY

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

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

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

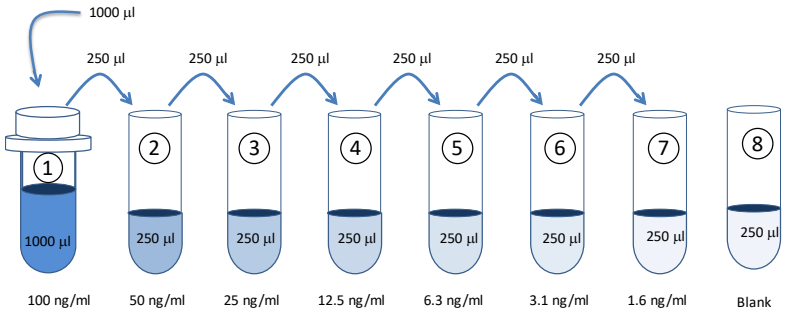
NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Coat Plate with Capture Antibody**: Dilute one vial of Capture Antibody with 5 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 50 µl of capture antibody solution to each well and incubate at 4°C overnight. Any leftover Capture Antibody Stock Solution may be stored at -20°C for future assays.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	8	0.8
4	17	1.7
6	25	2.5
8	33	3.3
10	42	4.2
12	50	5.0

2. **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/Secondary Antibody Dilution Buffer (Solution C) for the 100 ng/ml standard. Then serially dilute it with Solution C. For example, mix 250 µl of the standard (100 ng/ml) with an equal volume of Solution C 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. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



3. **Prepare Sample Dilutions:** Centrifuge the samples at 10,000 rpm for 5 minutes to remove insoluble materials in urine and serum samples. Dilute samples 1:500-1:100,000 with Solution C 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.

4. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out*.

5. **Add Standards and Samples**: Add 50 µl of Solution C (blank), standards, and samples to designated wells in duplicate and 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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Detection Antibody Solution**: Dissolve one vial of Detection Antibody in 5 ml of Sample/Standard/Secondary Antibody Dilution Buffer (Solution C). Alternatively, dissolve one vial of Detection Antibody in 50 µl of Solution C and dilute accordingly. Add 50 µl of detection antibody solution to each well and incubate at room temperature for 1 hour. Any remaining detection antibody stock solution can be stored at -20°C for use in a second assay

Strip #	Detection Antibody (µl)	Solution C (ml)
2	8	0.8
4	17	1.7
6	25	2.5
8	33	3.3
10	42	4.2
12	50	5.0

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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution by diluting 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.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add OPD Solution**: Dissolve one vial of OPD in 10 ml of Chromogen Dilution Buffer just prior to use. Add 50 µl of OPD solution to all wells immediately after washing the plate and incubate for 10 minutes at room temperature.

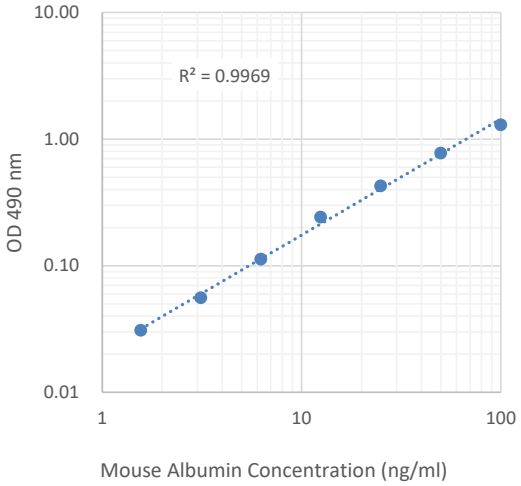
12. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

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.

CALCULATING 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 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 the Mouse Albumin Detection ELISA Kit



ASSAY VALIDATION

Table 1 - Reproducibility Data for the Mouse Albumin Detection ELISA Kit

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

* Known amounts of mouse albumin were added to a normal mouse urine pool and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer (Solution C).

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.’s ELISA FAQ for more information.

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