Rat Albumin Detection ELISA Kit

Catalog # 3020

For Research Use Only - Not Rat or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify rat albumin
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	3.5 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 (2.1-6.5%)/Inter-Assay (4.1-8.7%)/Spiking Test (91-94%)
NOTES:	

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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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) as 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 ELISA Kit (Cat # 3020) and a Rat Urinary Protein Assay Kit (Cat # 9040) depending on the purpose of the study.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Rat Albumin Standard (30201)	1 vial	100 ng, lyophilized	-20°C
Capture Antibody (30202)	1 vial	50 µl, 1 mg/ml	-20°C
Detection Antibody (30203)	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
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 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.

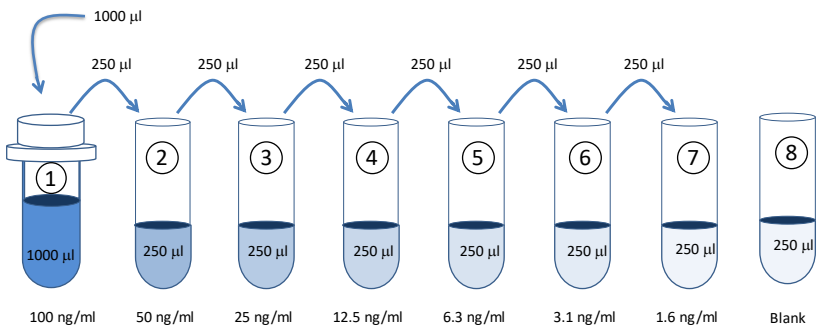
NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

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.



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

   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 C. For example, if samples are 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 C for a 1:20,000 dilution.

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 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.

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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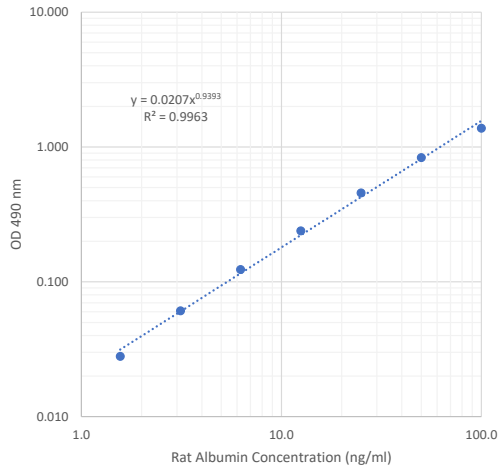
CALCULATING RESULTS

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 rat 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 rat albumin in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the Rat Albumin Detection ELISA Kit



ASSAY VALIDATION

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

Test	2.7 ng/ml	12 ng/ml	48 ng/ml
Intra-Assay CV (%)	5.6	6.5	2.1
Inter-Assay CV (%)	4.1	8.7	7.1
Spike Test* (%)	94%	94%	91%

* Known amounts of rat albumin were added to a normal rat 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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