Albumin (human) ELISA Kit

Item No. 501760

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401762	Albumin (human) ELISA Monoclonal Antibody	1 vial/100 dtn
401760	Albumin (human) HRP Tracer (6X)	1 vial/100 dtn
401404	Albumin (human) ELISA Standard (serum)	1 vial
401405	Albumin (human) ELISA Standard (urine)	1 vial
401703	Immunoassay Buffer C Concentrate (10X)	2 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400009	Goat Anti-Mouse IgG-Coated Plate	1 plate
400012	96-Well Cover Sheet	1 ea
400074	TMB Substrate Solution	2 vials/12 ml
10011355	HRP Stop Solution	1 vial/12 ml
400035	Polysorbate 20	1 vial/3 ml

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Albumin (human) ELISA Kit (Item No. 501760). This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.*, safety glasses, gloves, and lab coat) when using this material.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Albumin is a non-glycosylated and negatively charged protein that comprises 50% of total plasma protein content.[1] It is a globular protein that exists primarily in a reduced form, but also exists as mixed disulfides in complex with thiol-containing substances, such as cysteine and glutathione, in plasma. Urinary albumin exists in multiple molecular forms due to chemical modification by free radicals, oxidants, or proteolysis.[2] These modified forms are abundant in urine, but not plasma, due to selective renal tubular uptake and differential filtration. Albumin has roles in the maintenance of osmotic pressure, buffering blood pH, and the transport of hormones, fatty acids, metal ions, metabolites, and pharmaceuticals throughout the body. Serum levels of albumin have prognostic significance, exhibiting a negative correlation with all-cause mortality.[3,4] Plasma albumin levels are decreased and associated with poor prognosis and mortality in patients with cirrhosis.[3] Elevated levels of urinary albumin, both microalbuminuria and macroalbuminuria, are indicative of the development of diabetic nephropathy and non-diabetic kidney disease, associated with cardiovascular morbidity and mortality, and used as prognostic markers in various disease states.[5-7] The measurement of urinary albumin is well established as a clinical indicator of renal function.

About This Assay

Cayman's Albumin (human) ELISA Kit is a competitive assay that can be used for quantification of human albumin in plasma, serum, and urine. The assay uses two standards: serum albumin for plasma and serum measurements, and an albumin purified from human urine for the measurements in urine. The assay has a range of 7.8-1,000 ng/ml with a midpoint (50% B/B_0) of 90-130 and 50-80 ng/ml in serum and urine, respectively, and a sensitivity (80% B/B_0) of approximately 25 ng/ml in serum and 15 ng/ml in urine.

Principle of the Assay

This assay is based on the competition between native albumin and an albumin-horseradish peroxidase (HRP) conjugate (Albumin-HRP Tracer) for a limited amount of Albumin Monoclonal Antibody. Because the concentration of the Albumin-HRP Tracer is held constant while the concentration of native albumin varies, the amount of Albumin-HRP Tracer that is able to bind to the Albumin Monoclonal Antibody will be inversely proportional to the concentration of native albumin in the well. This antibody-albumin complex binds to goat anti-mouse IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and TMB Substrate Solution (which contains the substrate to HRP) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 450 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Albumin-HRP Tracer bound to the well, which is inversely proportional to the amount of free albumin present in the well during the incubation, as described in the equation:

$$\text{Absorbance} \propto \text{[Bound Albumin-HRP Tracer]} \propto 1/\text{[albumin]}$$

A schematic of this process is shown in Figure 1, see page 9.

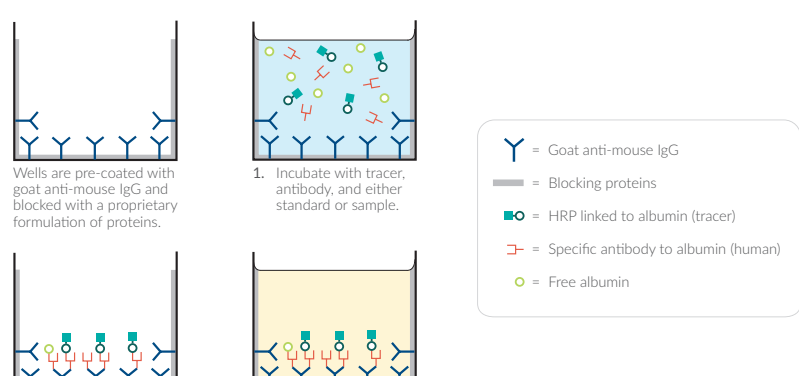


Figure 1. Schematic of the ELISA

Definition of Key Terms

Blk (Blank): background absorbance caused by TMB Substrate Solution and the HRP Stop Solution. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including the non-specific binding (NSB) wells.

TA (Total Activity): total enzymatic activity of the albumin HRP-linked tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a sample or standard well to the average absorbance of the maximum binding (B_0) wells.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn (Determination): one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. NOTE: It is normal for the concentrated buffers to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer C (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer C Concentrate (10X) (Item No. 401703) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X). *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Sample Preparation

This assay has been validated in human plasma, serum, and urine. See pages 14-16 for the validation data. Proper sample storage and preparation are essential for consistent and accurate results. Please read this section thoroughly before beginning the assay.

General Precautions

- All samples must be free of organic solvents prior to assay.
- Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.[6]

Plasma and Serum

Plasma and serum samples can be tested without any purification. It is recommended that plasma and serum samples be diluted at least 1:200,000 into Immunoassay Buffer C (1X) prior to testing in the assay.

Urine

It is recommended that urine samples be diluted at least 1:20 into Immunoassay Buffer C (1X) prior to testing in the assay.

NOTE: Urine samples should be standardized to creatinine levels in the urine. Cayman's Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701) is a quick, convenient method for measuring creatinine in urine.

Sample Matrix Properties

Linearity

Plasma and serum samples (unspiked) and a urine sample spiked with urinary albumin were serially diluted with Immunoassay Buffer C (1X) and evaluated for linearity using the Albumin (human) ELISA kit. The results are shown in the table below.

Sample	Dilution Factor	Concentration (µg/ml)	Dilutional Linearity (%)
Plasma	200,000	55,558	100%
	400,000	56,327	101%
	800,000	57,468	103%
	1,600,000	55,129	99%
Serum	200,000	57,958	100%
	400,000	60,027	104%
	800,000	60,113	104%
	1,600,000	60,675	105%
Urine	200	38.5	100%
	400	38.4	100%
	800	36.0	94%
	1,600	30.7	80%

Table 1. Dilutional linearity in plasma, serum, and urine

NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted/First observed concentration value in the dilution series, dilution adjusted)*100*

Spike and Recovery

Urine samples were spiked with different amounts of urinary albumin, diluted as described in the Sample Preparation section (see page 13), and analyzed using the Albumin (human) ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

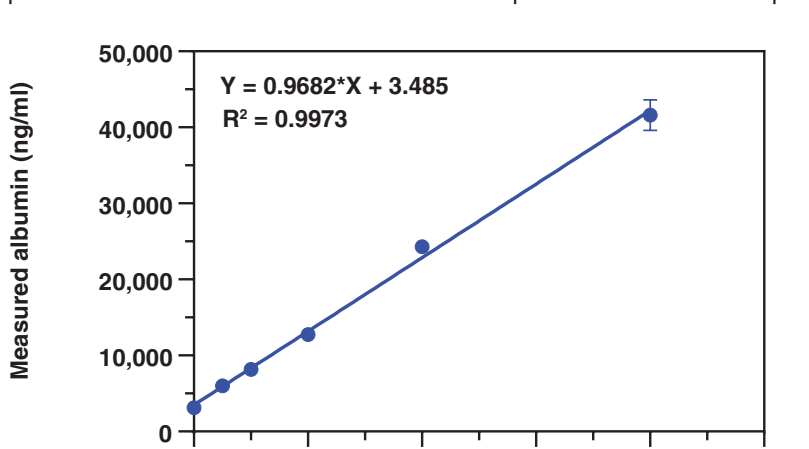


Figure 2. Spike and recovery of albumin in urine

Parallelism

To assess parallelism, plasma, serum, and urine were processed as described in the Sample Preparation section (see page 13), serially diluted with Assay Buffer (1X), and evaluated using the Albumin (human) ELISA Kit. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

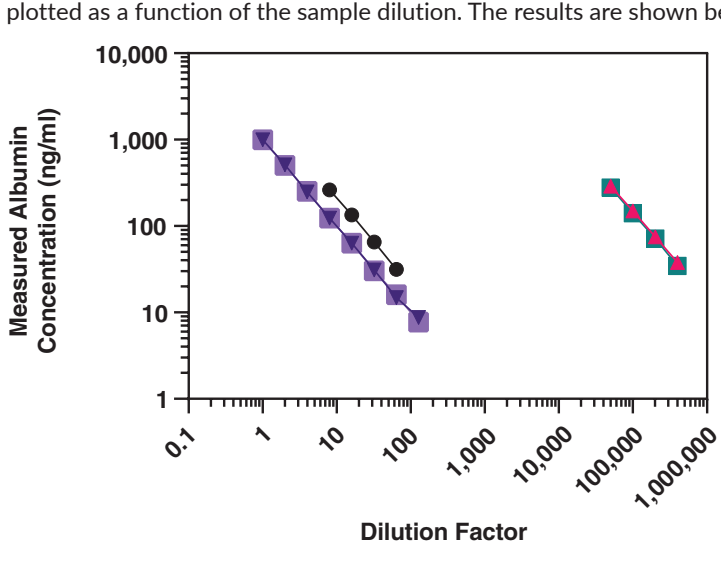


Figure 3. Parallelism of various matrices in the Albumin (human) ELISA Kit Due to the similarity of the standards and measurements in serum and plasma, the two standard curves and the graphs for serum and plasma overlap.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Albumin (human) ELISA Standard

Use the Albumin (human) ELISA Standard (serum) (Item No. 401404) for testing of serum and plasma samples and the Albumin (human) ELISA Standard (urine) (Item No. 401405) for testing of urine samples. Reconstitute the lyophilized Albumin (human) ELISA Standard (serum) or Albumin (human) ELISA Standard (urine) with 2 ml of Immunoassay Buffer C (1X), and mix gently. The concentration of this solution (the bulk standard) will be 10 µg/ml. The reconstituted standard will be stable for approximately four weeks when stored at 4°C.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl Immunoassay Buffer C (1X) to tube #1 and 500 µl Immunoassay Buffer C (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (10 µg/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. These diluted standards should be used within 24 hours.

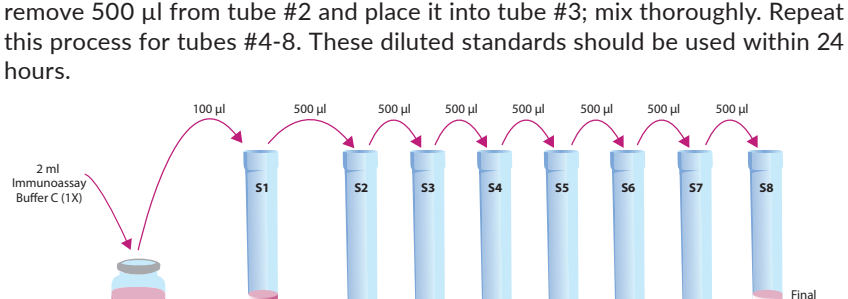


Figure 4. Preparation of the albumin standards

Albumin (human) HRP Tracer

Dilute the Albumin (human) HRP Tracer (Item No. 401760) with 5 ml of Immunoassay Buffer C (1X). Store the diluted Albumin (human) HRP Tracer at 4°C (*do no freeze!*) and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Albumin (human) ELISA Monoclonal Antibody

The Albumin (human) ELISA Monoclonal Antibody (Item No. 401762) is ready for use as provided. Store the antibody at 4°C and use by the kit expiration date. A 20% surplus of antibody has been included to account for any incidental losses.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk, two NSB, and three B_0 wells, and an eight-point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 5, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 22, for more details). We suggest recording the contents of each well on the template sheet provided (see page 33).

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	S1	S1	1	1	1	9	9	9	17	17	17
B	Blk	S2	S2	2	2	2	10	10	10	18	18	18
C	NSB	S3	S3	3	3	3	11	11	11	19	19	19
D	NSB	S4	S4	4	4	4	12	12	12	20	20	20
E	B_0	S5	S5	5	5	5	13	13	13	21	21	21
F	B_0	S6	S6	6	6	6	14	14	14	22	22	22
G	B_0	S7	S7	7	7	7	15	15	15	23	23	23
H	TA	S8	S8	8	8	8	16	16	16	24	24	24

Blk - Blank wells
TA - Total Activity well
NSB - Non-Specific Binding wells
B_0 - Maximum Binding wells
S1-S8 - Standard 1-8 wells
1-24 - Sample wells

Figure 5. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. **Immunoassay Buffer C (1X)**

   Add 100 µl of Immunoassay Buffer C (1X) to NSB wells. Add 50 µl of Immunoassay Buffer C (1X) to B_0 wells.

2. **Albumin (human) ELISA Standard**

   Add 50 µl from tube #8 to both of the lowest standard wells (S8). Add 50 µl from tube #7 to each of the next standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 50 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Albumin (human) HRP Tracer**

   Add 50 µl to each well *except* the TA and Blk wells.

5. **Albumin (human) ELISA Monoclonal Antibody**

   Add 50 µl to each well *except* the TA, NSB, and Blk wells.

Incubation of the Plate

Cover each plate with a 96-Well Plate Cover Sheet (Item No. 400012) and incubate for two hours at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X).
2. Add 175 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Mix 10 µl of the previously diluted tracer with 90 µl of Immunoassay Buffer C (1X). Add 5 µl of this solution to the TA wells.
4. Cover the plate with the 96-Well Cover Sheet and protect from light. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes.
5 Remove the plate cover being careful to keep TMB Substrate Solution from splashing on the cover. *NOTE: Any loss of TMB Substrate Solution will affect the absorbance readings.*
6. DO NOT WASH THE PLATE. Add 75 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively, a spreadsheet program can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the Blk wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool. Low or no absorbance from a TA well could indicate a dysfunction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* albumin concentration using linear (y) and log (x) axes and perform a four-parameter logistic fit.

Alternative Plot - The data can also be linearized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample by identifying the %B/B_0 on the standard curve and reading the corresponding values on the x-axis. *NOTE: Remember to account for any dilution of the original sample concentration prior to its addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference, which could be eliminated by purification.

NOTE: If there is an error in the B_0 wells it is possible to calculate sample concentrations by plotting the absorbance values and back calculating sample absorbance off the standard curve.

Performance Characteristics

Representative Data

The standard curves presented here are examples of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data from Tables 2 and 3, on pages 25 and 26, to determine the value of your samples.

Absorbance at 450 nm (30 minutes)

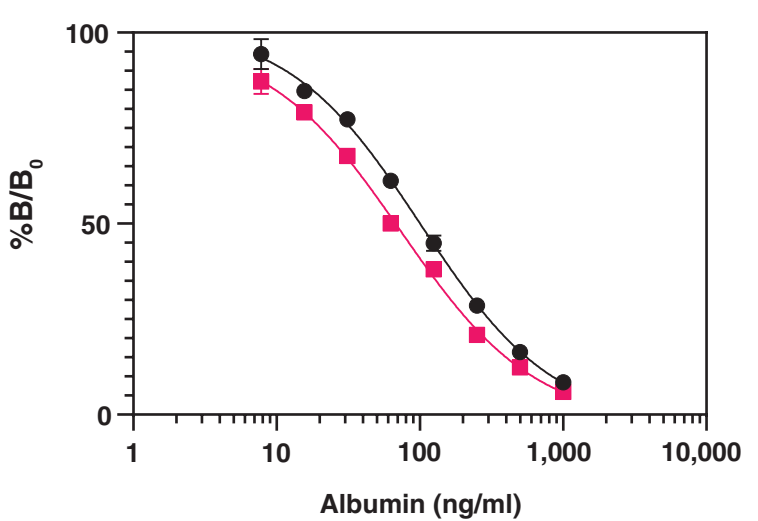
Analyte Standards (ng/ml)	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.003				
B_0	1.618	1.615			
1,000	0.097	0.094	5.9	5.5	6.1
500	0.201	0.198	12.4	1.9	4.7
250	0.338	0.335	20.9	2.7	3.6
125	0.615	0.612	38.0	3.6	3.6
62.5	0.810	0.807	50.1	7.0	3.7
31.25	1.095	1.092	67.7	9.1	2.8
15.63	1.279	1.276	79.1	15.4	6.7
7.81	1.409	1.406	87.2	14.2	8.8
TA	0.936	0.933			

Table 2. Typical results using the Albumin (human) ELISA Standard (urine)
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

Absorbance at 450 nm (30 minutes)

Analyte Standards (ng/ml)	Blk-Subtracted Absorbance	NSB-Corrected Absorbance	%B/B_0	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
NSB	0.004				
B_0	1.570	1.566			
1,000	0.136	0.132	8.5	2.8	6.0
500	0.261	0.257	16.4	3.0	5.7
250	0.451	0.447	28.5	2.6	2.2
125	0.706	0.702	44.8	4.3	2.5
62.5	0.963	0.959	61.2	6.1	5.7
31.25	1.214	1.210	77.3	6.2	10.1
15.63	1.331	1.327	84.7	17.4	23.2**
7.81	1.483	1.479	94.4	31.8**	27.8**
TA	1.054				

Table 3. Typical results using the Albumin (human) ELISA Standard (serum)
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve
**Evaluate data in this range with caution



Assay Range = 7.8-1,000 ng/ml
Sensitivity (defined as 80% B/B_0) = 24.9 ng/ml (serum) and 14.7 ng/ml (urine)
Mid-point (defined as 50% B/B_0) = 100.3 ng/ml (serum) and 67.6 ng/ml (urine)
Lower Limit of Detection (LLOD) = 8.6 ng/ml (serum) and 6.8 ng/ml (urine)

The sensitivity and mid-point were derived from the standard curves shown above. The standard was diluted with Immunoassay Buffer C (1X).

Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three human urine controls in a single assay.

Matrix Control (ng/ml)	%CV
1,679	10.0
2,053	8.0
2,945	7.7

Table 4. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three human urine controls in eight separate assays on different days.

Matrix Control (ng/ml)	%CV
1,841	9.1
2,777	8.9
1,755	11.2

Table 5. Inter-assay precision

Cross Reactivity:

This assay has been validated for measuring albumin in human plasma, serum and urine samples. Both mouse and rat albumin have been tested in this assay and found to be completely non-cross reactive.

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High NSB (>0.100 O.D.)	A. Poor washing B. Exposure of NSB wells to specific antiserum
Very low B_0	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Low sensitivity (shift in dose-response)	A. Standard is degraded or contaminated B. Dilution error in preparing standards
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present
Low signal in sample wells (below range of standard curve)	A. HRP inhibitors present: ensure that samples and buffers are free of HRP inhibitors, such as azide B. Sample requires further dilution
Only TA wells develop	A. Trace organic contaminants in the water source B. The tracer was not added to the wells

References

1. Quinlan, G.J., Martin, G.S., and Evans, T.W. Albumin: Biochemical properties and therapeutic potential. *Hepatology* **41(6)**, 1211-1219 (2005).
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4. Corti, M.C., Guralnik, J.M., Salive, M.E. *et al.* Serum albumin level and physical disability as predictors of mortality in older persons. *JAMA* **272(13)**, 1036-1042 (1994).
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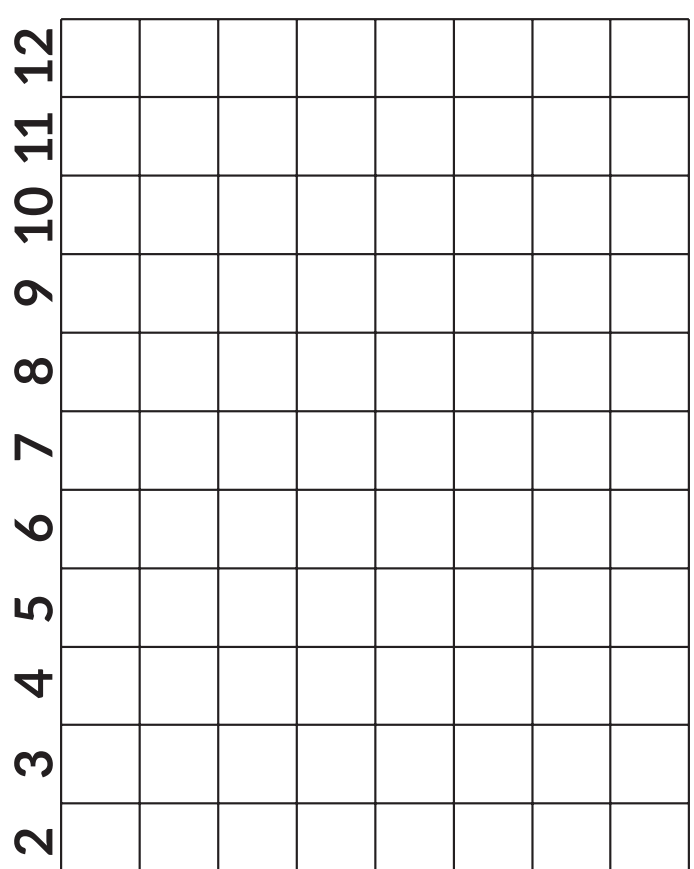
7. Brinkman, J.W., de Zeeuw, D., Duker, J.J., *et al.* Falsely low urinary albumin concentrations after prolonged frozen storage of urine samples. *Clin. Chem.* **51(11)**, 2181-2183 (2005).

Procedure	Blk	TA	NSB	B_0	Standards/ Samples
Reconstitute and mix	Mix all reagents gently				
Immunoassay Buffer C (1X)	--	--	100 µl	50 µl	--
Standards/Samples	--	--	--	--	50 µl
Albumin-HRP Tracer	--	--	50 µl	50 µl	50 µl
Albumin (human) ELISA Antibody	--	--	--	50 µl	50 µl
Seal	Seal the plate and tap gently to mix				
Incubate	Incubate plate for 2 hours at room temperature on an orbital shaker.				
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)				
Apply TMB Substrate Solution	175 µl	175 µl	175 µl	175 µl	175 µl
TA - Apply Tracer additionally diluted 1:10	--	5 µl	--	--	--
Seal	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker, protected from light				
HRP Stop Solution	75 µl	75 µl	75 µl	75 µl	75 µl
Read	Read absorbance at 450 nm				

Table 6. Assay summary



NOTES

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