Cat. # MK132

For Research Use

TaKaRa

Human Albumin EIA Kit

Product Manual

v201607

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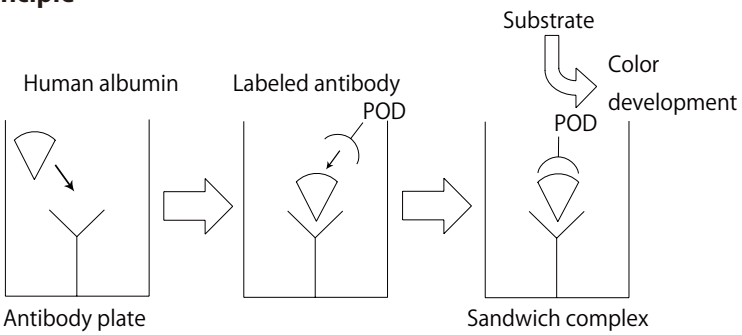
I. Description

Albumin is a protein of about 66,000 Daltons present in blood at a very high concentration. In addition to maintaining blood osmotic pressure, it is also known to play a role in transporting fatty acids and a variety of other substances by forming complexes with them.

Albumin, which is hepatically synthesized and renally removed, serves as an indicator of liver function. Albumin has attracted attention as a potential early diagnostic marker to detect diabetic nephropathy since studies have shown that a slight trace of albumin in urine was detected before urinary protein increased.

This quantification kit using a human albumin-specific monoclonal antibody can be utilized as a simple tool to monitor albumin levels in human serum and body fluids, among other purposes.

II. Principle



III. Components

(1) Antibody Coated Microtiter plate Anti-Human albumin monoclonal antibody (96 wells: 8 wells x 12 strips)	1 plate
(2) Antibody - POD Conjugate (lyophilized) Peroxidase-labeled anti-human albumin monoclonal antibody	For 11 ml
(3) Standard (lyophilized) human albumin, 160 ng	For 1 ml
(4) Sample Diluent 25% Block Ace-containing PBS (with preservative)	11 ml x 2
(5) Substrate Solution (TMBZ) 3, 3', 5, 5'-Tetramethylbenzidine Solution	12 ml

IV. Storage 4℃

V. Materials required but not Provided

- Wash and Stop solution for ELISA without Sulfuric Acid (Cat. #MK021)
  Contains wash solution (10X PBS, 50 ml x 5 tubes; Tween 20, 3 ml) and reaction stop solution (60 ml).
  - ＊ This product is a stop solution for peroxidase reactions without 1N sulfuric acid.
  - ＊ 1N sulfuric acid can be used as a stop solution. Handle 1N sulfuric acid with caution.
- Pipette, micropipette, and tips
- Microplate reader (capable of measuring absorbance of up to 3.5 when set to 450 nm)

VI. Intended Use

In vitro enzyme immunoassay for quantitative determination of human albumin in human serum and body fluid samples.

VII. Protocol

1. Sample

- Samples may be stored up to 12 hours at 2 - 10℃. If the assay will be performed longer than 12 hours after sample preparation, then store samples frozen at -20℃.
- For sample dilutions, refer to the assay examples given later in the protocol. Dilute samples that may contain a high level of albumin with Sample Diluent (4).
- The recommended dilution for human serum samples is 1 : 10^6 or greater.

2. Reagent preparation

- Antibody Coated Microtiter Plate
  Allow the (1) Anti-Human albumin monoclonal antibody plate to reach room temperature unopened in its package before use.
- POD-labeled Antibody Solution
  Reconstitute (2) Antibody - POD Conjugate with 11 ml of distilled water.

  Once reconstituted, it is stable for up to 1 week at 4℃. For longer storage, freeze at -20℃, at which it is stable for up to 1 month. Once thawed, it may not be returned to frozen storage.
- Standard Solution
  Add 1 ml of distilled water to the (3) Standard to reconstitute the Standard (160 ng/ml). Dilute it with (4) Sample Diluent before use to prepare fresh serial dilutions of Standard Solution at concentrations of 160, 80, 40, 20, 10, 5, and 2.5 ng/ml. Use Sample Diluent as the 0-concentration standard.

  The Albumin Standard Solution (160 ng/ml) is stable for up to 1 week after preparation when stored at 4℃, or for up to 1 month at -20℃.
- Substrate Solution
  Return (5) Substrate Solution (TMBZ) to room temperature before use. It is supplied ready to use. Check before use that the Substrate Solution has not developed a dark blue color. A reaction with metal ions will result in coloration; make sure it is not contaminated with any tap water.

  If the Substrate Solution will be used for several assays, divide it into aliquots of the required volume in advance.

- Stop solution
  Use the Stop solution included in Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021) directly.
  ＊ Because this is highly viscous, mix well using a plate mixer after its introduction.
- PBS for Wash
  Dilute 10X PBS in the Wash and Stop Solution for ELISA without Sulfuric Acid with distilled water to obtain a 1X solution.
  96 reactions requires approximately 300 ml of wash solution.

3. Procedure

Assay samples in duplicate.
Return each reagent in the kit and samples to room temperature and make sure solutions are mixed uniformly without creating bubbles before use.

Note: Prepare serial dilutions of the Standard Solution and samples in a separate 96 well plate in advance so that they can be added to the antibody-plate quickly (within 5 minutes) using an 8-channel pipette or similar apparatus.

1. Add prepared samples (100 μl/well)to the Antibody Coated Plate. In order to provide highly reliable results, it is recommended to place serial dilutions of the Standard Solution (100 μl/well) in the 1st and 12th rows. Perform this reaction for 2 hours at room temperature (20 - 30℃); incubation at 37℃ may compromise antigenicity. [First reaction]
2. Remove reaction mixtures from wells, discarding the liquid. Wash wells 3 times with Washing Buffer (100 μl/well). Remove excess Washing Buffer and then add 100 μl of the Antibody-POD Conjugate Solution per well using an 8-channel pipette and allow to react for 1 hour at room temperature (20 - 30℃). [Second reaction]
3. Remove reaction mixtures from wells, discarding the liquid. Wash wells 4 times with Washing Buffer (100 μl/well). Remove excess Washing Buffer and then add 100 μl of (5) Substrate Solution (TMBZ) per well using an 8-channel pipette and allow to react for 10 - 15 minutes at room temperature (20 - 30℃). [Third reaction]
4. Add 100 μl of Stop Solution＊ to each well in the same order as for (5) Substrate Solution (TMBZ) to stop the reaction. Then mix well.
   ＊: As the viscosity of Stop Solution is high , it is necessary to mix well using the plate mixer after adding to the plate.
5. Use distilled water as a control to make zero adjustment and measure absorbance at 450 nm.
   The color is stable for up to 1 hour after reaction termination.
6. Plot a standard curve based on the results obtained from the Standard Solutions (with concentration as x-axis and absorbance as y-axis) and use it to determine the corresponding concentrations of Human albumin based on the sample's absorbance.

Note:

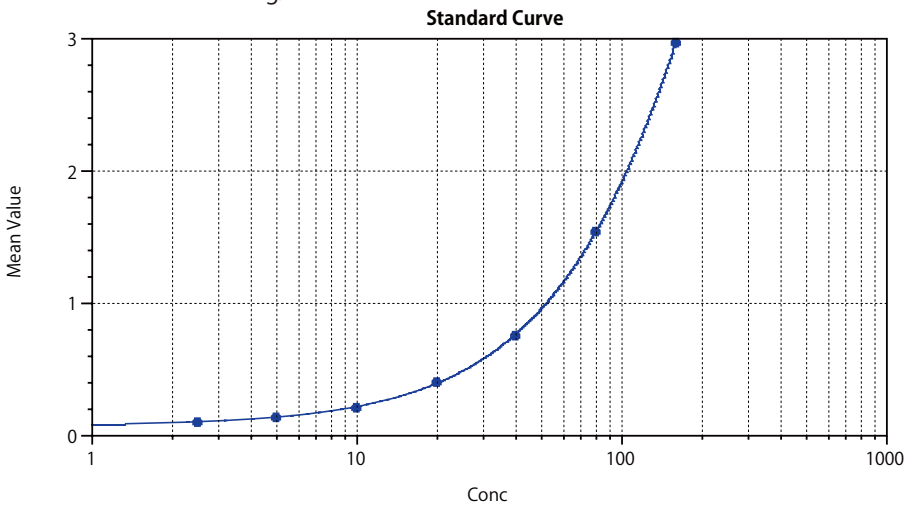
- Cover the plate with film or the like to prevent evaporation of solutions during reactions at room temperature or in an incubator.
- It is recommended that the Washing Buffer be completely discarded to get rid of the residual fluid.

VIII. Performance

1. Standard curve

The following shows a typical standard curve of this kit as an example.
The standard curve for calculation needs to be established in each assay.

Limit of detection: 2.5 ng/ml



4-P Fit: $y = (A - D)/\{1 + (x/C)^B\} + D$:

A	B	C	D	R^2
0.0707	1.17	475	13.3	1

Std (Standards: Conc vs MeanValue)
Curve Fit Option - Fixed Weight Value

Human albumin (ng/ml)	160.0	80.0	40.0	20.0	10.0	5.0	2.5	0.0
A_{450}	2.961	1.538	0.754	0.396	0.211	0.139	0.102	0.065

Color development: 15 min

2. Reproducibility

<Intra-assay precision (n=8)>

The reproducibility test was performed with 8 replicates, using 3 different concentrations of human serum.

Sample	Average (ng/ml)	CV (%)
Control A	125.4	2.62
Control B	63.4	3.00
Control C	28.1	3.74

<Inter-assay precision (n=3)>

The reproducibility test was performed in triplicates, by assaying 3 different concentrations of control over 3 days.

Sample	Average (ng/ml)	CV (%)
Control A	129.3	7.44
Control B	65.3	7.94
Control C	28.5	4.42

3. Recovery test

Equal volumes of samples in different concentrations were combined and assayed. The result of each mixture was compared with the theoretical value to detemine the recovery rate.

Sample A	Sample B	A+B (Theoretical Value)	A+B (Assay Result)	Recovery Rate (%)
29.5	21.5	25.5	25.0	98
64.6	21.5	43.1	42.3	98
64.6	29.5	47.1	49.2	105
95.2	21.5	58.3	53.9	92
95.2	29.5	62.4	58.5	94
126.9	21.5	74.2	75.7	102
126.9	29.5	78.2	76.2	97
95.2	64.6	79.9	79.1	99
146.5	29.5	88.0	83.2	94
146.5	21.5	84.0	87.3	104
126.9	64.6	95.8	95.7	100
95.2	126.9	111.1	108.6	98
146.5	64.6	105.6	114.9	109
146.5	95.2	120.9	116.0	96
146.5	126.9	136.7	131.2	96

(Unit: ng/ml)

4. Cross reactivity with serum samples from various species

Cross reactivity with serum albumins from various species were studied. The standard concentration of albumin in animal serum samples is estimated to be 30 - 50 mg/ml.

Serum Species (Dilution Ratio)								
1 x 10^2	1 x 10^5	2 x 10^5	1 x 10^2	1 x 10^5	2 x 10^5	1 x 10^2	1 x 10^5	2 x 10^5
Human donor No. 1			Bovine			Rabbit		
Human donor No. 2			Horse (male)			Chicken		
Human donor No. 3			Porcine			Goose		
Human donor No. 4			Human (pooled serum)			Turkey		
Human donor No. 5			Dog			Domestic duck		
Human donor No. 6			Guinea Pig			Cynomolgus No. 1		
			Rat			Cynomolgus No. 2		
			Mouse			Goat		
+4 over	0.2	0.2	0.1	ND	ND	ND	ND	ND
+4 over	0.2	0.1	0.3	ND	ND	ND	ND	ND
+4 over	0.2	0.1	+4 over	ND	ND	ND	ND	ND
+4 over	0.3	0.2	+4 over	0.3	0.1	ND	ND	ND
+4 over	0.3	0.1	ND	ND	ND	ND	ND	ND
+4 over	0.2	0.1	ND	ND	ND	+4 over	ND	ND
			0.1	ND	ND	+4 over	ND	ND
			0.1	ND	ND	ND	ND	ND

ND : A_{450} = 0.050 or less +4 over : A_{450} = 4.0 or more

Result: Cross reactivity was observed with bovine, horse (male), porcine, rat and mouse serum samples, but only at low serum dilution ratios. In contrast, albumin was detected in highly diluted human serum samples (dilution ratio = 2 x 10^5).

IX. Experimental Examples

1. Culture Supernatant

- Human liver cancer cells (Hep 3B: Dainippon Sumitomo Pharma Co., Ltd.) were cultured in antibiotic-containing RPMI1640 medium supplemented with 10% FCS (Sigma-Aldrich).
- Concentrations of human albumin in the culture supernatant or in its 5-fold dilution were monitored over time.

Sample	Medium	Day 2	Day 3	Day 4	Day 7	Day 10	Day 15
x 1	0.176	0.913	2.981	3.263	over	over	over
x 5	0.100	0.220	1.090	1.370	3.389	over	over

over : A_{450} beyond the top of detection range (A_{450} > 4.0) (A_{450})

2. Human Urine Sample

Human urine samples were diluted 1 : 5 - 1 : 5^7 and assayed for albumin concentration.

Dilution ratio	x 5^1	x 5^2	x 5^3	x 5^4	x 5^5	x 5^6	x 5^7
Age 0, Male	over	2.963	0.908	0.226	0.08	0.063	ND
Age 69, Male	3.645	3.676	2.955	0.898	0.339	0.245	0.197

ND : A_{450} below limit of detection over : A_{450} above the limit of detection (A_{450})

X. Precautions

1. Do not mix/use kits or reagents from different lots.
2. Do not expose reagents to strong light during storage or reactions.
3. Use pipettes free of metal when handling (5) Substrate Solution (TMBZ).
4. Exercise care to prevent (5) Substrate Solution (TMBZ) from coming into contact with hands or mucous membranes.
5. Do not use (5) Substrate Solution (TMBZ) that has developed color.
6. Each reaction varies subject to length of time and temperature. Therefore, a new standard curve must be generated for each assay.
7. Handle blood samples with great care.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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