Cat. # MK133

For Research Use

TaKaRa

Mouse Albumin EIA Kit

Product Manual

v201509Da

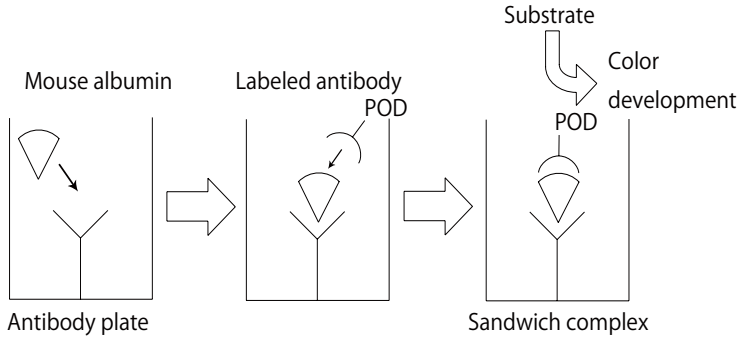
Table of Contents

I. Description

Albumin is a protein of about 66 kDa that is present in blood at very high concentrations. In mouse embryonic development, the early liver primordium begins to form around embryonic day 9, and the surrounding liver parenchymal cells, connective tissue, and associated vascular system tissues (portal vein, artery, vein, and sinusoidal tissues) develop during fetal days 13 - 17. Albumin and alpha-fetoprotein (AFP) produced by fetal hepatic parenchymal cells are both early markers of hepatic differentiation in the endoderm, but AFP is the major serum protein until birth. Shortly before birth, there is a switch from AFP production to albumin production, and the concentration of albumin gradually increases as the concentration of AFP decreases.

This product is a sandwich-type mouse albumin assay kit that uses two rat monoclonal antibodies generated against mouse albumin. The kit makes it possible to easily measure mouse albumin *in vitro* and *in vivo*. Since the assay is performed using monoclonal antibodies, it provides excellent specificity because these antibodies do not cross-react with albumin from other species.

II. Principle



III. Components

(1) Antibody Coated Microtiter plate — 1 plate (96 wells: 8 wells x 12 strips)
Anti-mouse albumin monoclonal antibody

(2) Antibody - POD Conjugate (lyophilized) — For 11 ml
Peroxidase-labeled anti-mouse albumin monoclonal antibody

(3) Standard (lyophilized) — For 1 ml
Albumin from mouse plasma, 640 ng

(4) Sample Diluent — 11 ml x 2
25% Block Ace containing PBS (with preservative)

(5) Substrate Solution (TMBZ) — 12 ml
3, 3', 5, 5'-Tetramethylbenzidine Solution

IV. Materials required but not Provided

- Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021)
  - Wash Solution [10X PBS (50 ml x 5); Tween 20 (3 ml)], and Stop Solution* (60 ml)
    *: This product is a peroxidase reaction stop solution that does not include 1 N sulfuric acid (which can also be used as a stop solution).
- Distilled water
- Microtiter plate reader that can measure absorbance values up to 3.5 at 450 nm

V. Storage 4℃

VI. Intended Use

In vitro enzyme immunoassay for quantitative determination of mouse albumin in blood, urine, cell extracts, and cell culture supernatants.

VII. Protocol

1. Sample guidelines

- Use with mouse plasma, serum, urine, cell extract, cell culture supernatant, etc.
- Assay reacts with mouse antigen, but does not cross-react with other species.
- Samples may be stored up to 12 hours at 2 - 10℃. If the assay will be performed more than 12 hours after sample preparation, then store samples frozen at -20℃.
- Dilute samples that may contain a high level of albumin with Sample Diluent (4).
- For blood or ascites fluid, assay samples that have been diluted at least 4,000-fold. When performing the dilution, first prepare a 100-fold dilution, then perform two serial dilutions that result in an additional 40-fold dilution.
- For urine, assay samples ranging from undiluted to 10-fold diluted.

2. Reagent preparation

- Antibody Coated Microtiter Plate

  Allow the (1) Anti-Mouse albumin monoclonal antibody plate to warm to room temperature unopened in its package, just before use.

- Labeled Antibody Solution

  Reconstitute (2) Antibody - POD Conjugate with 11 ml of distilled water, making sure it is completely dissolved.

  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 it to a concentration of 640 ng/ml. Dilute with (4) Sample Diluent before use to prepare fresh serial dilutions of Standard Solution at concentrations of 640, 320, 160, 80, 40, 20, and 10 ng/ml. Use Sample Diluent as the 0-concentration standard.

  The Albumin Standard Solution (640 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. Make sure that the Substrate Solution has not turned dark blue before use. A reaction with metal ions will result in a change in color, so make sure it is not contaminated with tap water.

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

- Stop Solution

  The Stop Solution in Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021) is recommended.

  * The solution is highly viscous, so mix it thoroughly with a plate mixer or a similar device.

- Wash Buffer (PBS)

  Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021) contains 10X PBS (50 ml). Dilute with distilled water to a volume of 500 ml and mix thoroughly.

3. Procedure

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

1. Add prepared samples and standards (100 µl/well) to the Antibody Coated Plate in duplicate. Incubate for 1 hour at room temperature (20 - 30℃). (First reaction)

   Note: Prepare samples and serial dilutions of the Standard Solution 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.

2. Remove the reaction mixtures from the wells, discarding the liquid. Wash wells 3 times with PBS (100 µl/well). Remove PBS and then add 100 µl of the Labeled Antibody 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 the antibody solution from the wells, discarding the liquid. Wash wells 4 times with PBS (100 µl/well). Remove excess PBS and then add 100 µl of (5) Substrate Solution (TMBZ) per well using an 8-channel pipette, and allow to react for 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 the reactions well using the plate mixer after adding this solution to the plate.

5. Use distilled water as a control to make a zero adjustment and measure the 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 mouse albumin based on the absorbance of the samples.

VIII. Performance

1. Standard curve

The following example shows a typical standard curve generated using this kit.
A new standard curve needs to be generated for each assay.

Limit of detection: 10 ng/ml

Mouse albumin (ng/ml)	640	320	160	80	40	20	10	0
A_{450}	3.111	2.192	1.313	0.770	0.481	0.268	0.183	0.089

2. Reproducibility

<Intra-assay precision>

The reproducibility test was performed using 3 different concentrations of mouse serum.

Sample (n=19)	Average (ng/ml)	CV (%)
Control A	333.1	7.0
Control B	130.8	7.7
Control C	61.1	7.8

<Inter-assay precision>

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

Sample (n=3)	Average (ng/ml)	CV (%)
Control A	337.2	8.1
Control B	132.8	5.9
Control C	61.7	9.6

3. Recovery test

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

(Unit: ng/ml)

Sample A	Sample B	Theoretical Value (A+B)/2	Measured Value	Recovery Rate (%)
318.7	124.6	221.7	213.9	96.5
318.7	68.5	193.6	229.4	118.5
124.6	68.5	96.6	96.7	100.1
320.0	160.0	240.0	240.3	100.1
160.0	80.0	120.0	108.5	90.4
80.0	318.7	199.4	186.2	93.4
40.0	124.6	82.3	73.8	89.6

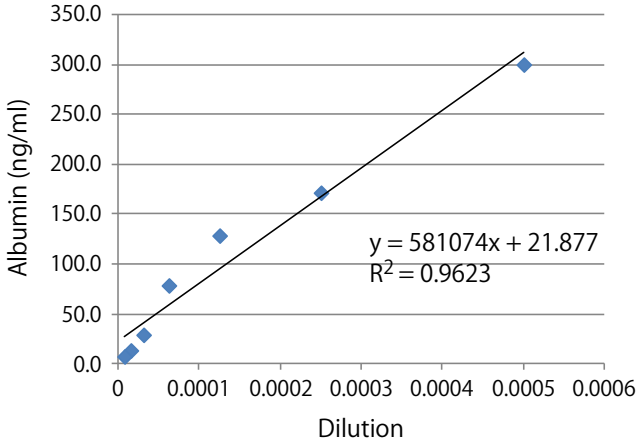
Result: Recovery rate was between 89.6 and 118.5%.

IX. Experimental Examples

1. Dilution curve

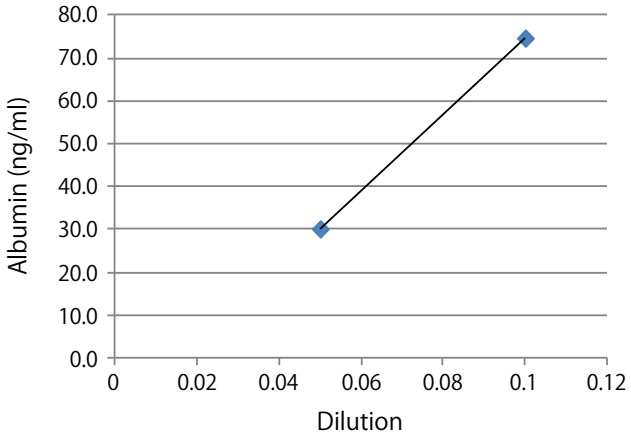
<Pooled mouse serum>

Dilution		Mouse albumin (ng/ml)
x 2,000	(0.0005)	300.5
x 4,000	(0.00025)	171.8
x 8,000	(0.000125)	128.8
x 16,000	(0.0000625)	78.7
x 32,000	(0.0000313)	29.1
x 64,000	(0.0000156)	13.4
x 128,000	(0.0000078)	7.4



<Mouse urine>

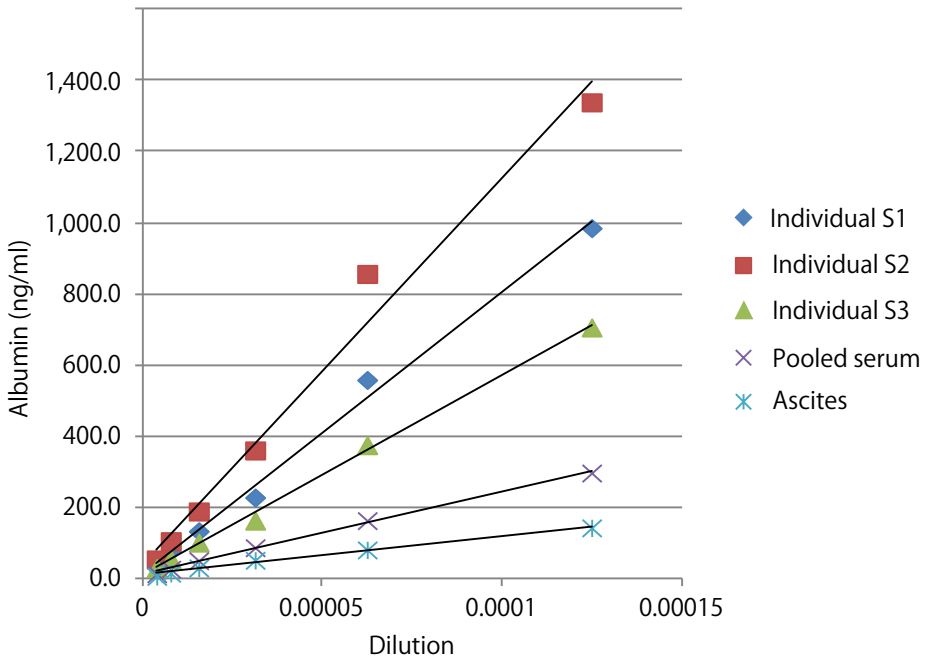
Dilution		Mouse albumin (ng/ml)
x 10	(0.1)	74.7
x 20	(0.05)	30.2



<Individual serum and ascites samples>

The albumin concentration in individual mouse sera, pooled mouse serum and ascites were determined by preparing a dilution series starting with a 2,000-fold dilution.

Dilution		Individual C57BL6			ICR 8W	Scid
		S1 (ng/ml)	S2 (ng/ml)	S3 (ng/ml)	Pooled serum (ng/ml)	Ascites (ng/ml)
x 2,000	(0.0005)	1047.9	1152.5	990.5	619.1	292.9
x 4,000	(0.00025)	1395.9	1370.5	1012.1	464.2	226.7
x 8,000	(0.000125)	984.6	1338.6	705.3	295.5	141.2
x 16,000	(0.0000625)	557.4	855.8	374.0	161.5	79.2
x 32,000	(0.0000313)	226.6	359.1	161.7	85.4	49.9
x 64,000	(0.0000156)	131.7	187.3	99.9	48.7	28.6
x 128,000	(0.0000078)	75.7	103.7	53.2	25.0	13.5
x 256,000	(0.0000039)	40.4	52.3	28.3	12.0	4.6



X. Precautions for Use

1. Do not mix/use kits or reagents from different lots.
2. Do not expose reagents to strong light during storage or when performing reactions.
3. Use metal-free pipettes when handling (5) Substrate Solution (TMBZ).
4. Do not allow (5) Substrate Solution (TMBZ) to come into contact with hands or mucous membranes.
5. Do not use (5) Substrate Solution (TMBZ) that has developed color.
6. Results vary between different sets of reactions, due to differences in the reaction time and temperature. Therefore, a new standard curve must be generated for each assay.
7. Handle blood samples with great care.

XI. Related Products

Wash and Stop Solution for ELISA without Sulfuric Acid (Cat. #MK021)
Anti-Mouse Albumin, Monoclonal (Clone M-Alb 151-1) (Cat. #M234)
Human Albumin EIA Kit (Cat. #MK132)