

Human L-FABP Assay Kit 96 Test

INTRODUCTION

This human type L-FABP ELISA kit is designed for the quantitative determination of L-type fatty acid binding protein(L-FABP) in human urine. L-FABP is a low molecular soluble protein (about 14kDa) expressed in the proximal convoluted tubule peculiarly in the kidney, and physiologically it is thought that L-FABP plays an important role in energy and lipid metabolism in the convoluted tubule where the function of re-absorption of the kidney is borne.

It is expected that L-FABP is useful as a prognosis marker in kidney diseases because it is induced and excreted into urine in response to stresses by the protein urea and the minute blood stream trouble, etc. that appear in the kidney disease extension.

This product is a sandwich type ELISA kit that uses the monoclonal antibodies that specifically recognizes human type L-FABP, and a stable and highly sensitive measurement of L-FABP in urine is possible with this kit. Moreover, the L-FABP Antibody Coated Microplate can be separated for measurement of a small number of specimen materials.

PRINCIPLE

The procedure described here is an ELISA(Enzyme-Linked-Immuno-Sorbent Assay) of 2-step sandwich method.

L-FABP Standard or urine samples are firstly treated with Pretreatment Solution, and transferred into L-FABP Antibody Coated Microplate containing Assay Buffer and incubated. During this incubation, L-FABP in the reaction solution binds to the immobilized antibody. After washing, the 2nd Ab-POD Conjugate is added as the secondary antibody and incubated, thereby forming sandwich of the L-FABP antigen between the immobilized antibody and conjugate antibody.

After incubation, the plate is washed and added with Substrate for enzyme reaction, and then color develops according to the L-FABP antigen quantity. The optical density is measured using a microplate reader, and a calibration curve is prepared based on the obtained optical density, thereby determining the L-FABP concentration.

MEASUREMENT RANGE

3 ~ 400 ng/mL

INTENDED USE

This kit is capable of the quantitative determination of human L-FABP in urine.

KIT COMPONENT

1. L-FABP Antibody Coated Microplate	96Well x 1
2. Pretreatment Microplate	96Well x 1
3. Pretreatment Solution	6mL x 1
4. Assay Buffer	12mL x 1
5. The 2 nd Ab-POD Conjugate	12mL x 1
6. Substrate	2
7. Substrate Diluent	12mL x 2
8. Wash Agent(x40 concentrate)	50mL x 1
9. Stop Solution	12mL x 1
10. Standard Diluent(0ng/mL)	2.5mL x 1
11. L-FABP Standard(400ng/mL)	0.5mL x 1

OPERATION MANUAL

1. Instruments and Equipments required

- Micropipette: adjustable to 20uL, 50uL
- Multichannel micropipette: adjustable to 50uL, 100uL
- Graduated cylinder: 2,000mL
- Microplate reader: wave length of 492nm(630nm)
- Plate Seal(attached in the kit)
- Plate mixer

2. Preparation of wash solution

Dilute whole volume of "8.Wash Agent" with distilled water to make 2,000mL.

3. Operation

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Prepare a standard curve while measuring test samples.

1) Preparation of L-FABP Standards

- a) As shown in Fig.1, use the first column(A1 ~ H1wells) of "2.Pretreatment Microplate" for the preparation.
- b) Add 50uL of "10.Standard Diluent(0ng/mL)"to B1 to H1wells of "2.Pretreatment Microplate" respectively.
- c) Add 50uL of "11.L-FABP Standard(400ng/mL)" into A1well.
- d) Also, add 50uL of "11.L-FABP Standard(400ng/mL)" to B1well and mix well gently(ten times pipetting). Then add 50uL of the solution to C1well and mix it as well.
- e) Carry out such the doubling dilution to G1well continuously one by one and remove 50uL of the solution from G1well.

2) Pretreatment

- a) After preparation of L-FABP Standards, add 50uL of sample specimens into the other wells(2A, 2B,...) of "2.Pretreatment Microplate".
- b) Add 50uL of "3.Pretreatment Solution" to all wells with L-FABP Standards and the samples specimens. Seal the plate and stir it for more than 5 minutes.

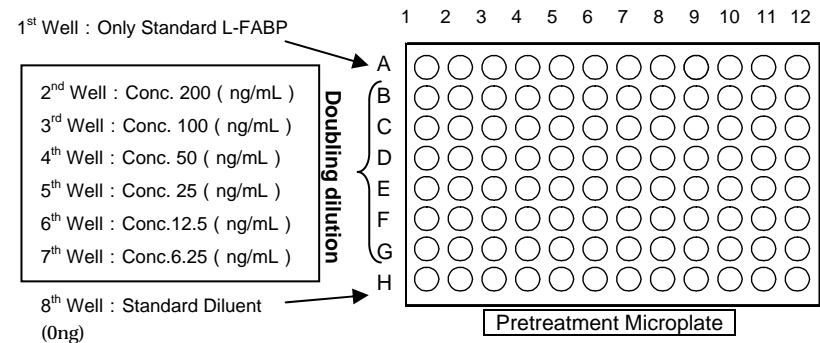


Fig.1 Example of operating pretreatment

- 3) Set the strips of "1. L-FABP Antibody Coated Microplate" (two strips for standard + strips for specimens) from left side(1,2...) in the plate holder, and add 100uL of "4.Assay Buffer" in each wells.
- 4) Pipette the standard solution from each well in the first column of "2. Pretreatment Microplate" and add the standard solution(20uL/well) to respective two wells in the first two strips of "1. L-FABP Antibody Coated Microplate".
- 5) Pipette 20uL of the pretreated sample specimen from "2. Pretreatment Microplate" and add the solution to respective wells after third strips of "1. L-FABP Antibody Coated Microplate".
- 6) Seal "1. L-FABP Antibody Coated Microplate" and stir it for 5 minutes with the plate mixer, and then incubate "1. L-FABP Antibody Coated Microplate" for 55 minutes at room temperature(20 ~ 28°C).
- 7) After incubation, throw away the liquid from "1.L-FABP Antibody Coated Microplate".
- 8) Wash each well of "1. L-FABP Antibody Coated Microplate" with wash solution (350uL/well). Then, fill each well with wash solution and remove wash agent completely from "1. L-FABP Antibody Coated Microplate" by snapping it. This procedure must be repeated 3 times. Then, remove the remaining liquid from all wells completely by snapping "1. L-FABP Antibody Coated Microplate" onto paper towels.
In case of using a plate washer, wash it with 350uL 3 times.
- 9) Pipette 100uL of "The 2nd Ab-POD Conjugate" into the wells of test samples, standards, and test sample blank.
- 10) Seal the plate and stir it for 5 minutes with the plate mixer, and then incubate the plate for 55 minutes at room temperature(20 ~ 28°C).
- 11) Substrate solution: One Substrate tablet is reconstituted in 12mL of "7.Substrate Diluent (1 bottle)" and used as a substrate solution. The substrate solution is prepared 15 minutes prior to use.
- 12) After incubation of step 10), throw away the liquid, and then wash the plate 3 times in the same manner as step 8).
- 13) Pipette 100uL of Substrate solution into the wells.
- 14) Seal the plate and stir it for 5 minutes with the plate mixer, and then incubate the plate for 25 minutes at room temperature(20 ~ 28°C) in the dark.
- 15) Pipette 100uL of "9.Stop Solution" into the wells. Mix the liquid by tapping the side of the plate.
- 16) Remove any dirt or drop of water on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, read the absorbance of each well at 492nm using a microplate reader. If a dual wavelength plate reader is available, set the test wave length at 492nm and reference at 630nm.
- 17) Make a standard curve based on the absorbance of standard L-FABP and calculate the amount of L-FABP in the specimen.

Fig.2 Operation Protocol

	Test Sample	Standard	Blank
Pretreatment	Test Sample 50uL	L-FABP Standard 50uL	Standard Diluent (0ng/mL)50uL
	Pretreatment reagent 50uL		
	Mix for more than 5 minutes by Plate Mixer after sealing plate		
Assay Buffer	100uL	100uL	100uL
Pretreated samples	20uL	20uL	20uL
	Mix for 5 minutes by Plate Mixer after sealing plate		
	Incubate for 55 minutes at room temperature		
	Wash 3 times		
Labeled antibody	100uL	100uL	100uL
	Mix for 5 minutes by Plate mixer and after sealing plate		
	Incubate for 55 minutes at room temperature		
	Wash 3 times		
Substrate Solution	100uL	100uL	100uL
	Mix for 5 minutes by Plate mixer and after sealing plate		
	Incubate for 25 minutes at room temperature with light shielding		
Stop Solution	100uL	100uL	100uL
	Tap the plate for mixing, and then measuring wavelength 492nm(reference wavelength 620nm) within 30 minutes after addition of Stop solution.		

SPECIAL ATTENTION

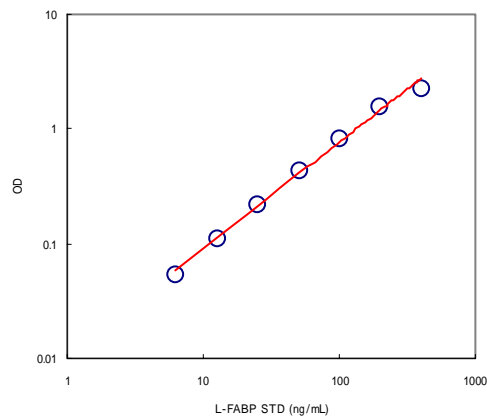
1. Test samples should be measured soon after collection. For storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2. Test samples should be diluted with Standard Diluent if required.
3. Put an unused L-FABP Antibody Coated Microplate in a bag and preserve it in refrigerator until next use.
4. Duplicate measurement of test samples and standard is recommended.
5. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
6. Use only Wash Agent contained in this kit for washing L-FABP Antibody Coated Microplate. Insufficient washing may lead to failure in measurement.
7. Don't tight seal with cap, because the substrate solution will be sparkling. Not stare or reuse the used solution.
8. Measurement should be done within 30 minutes after addition of "Stop Solution".

CALCURATION OF TEST RESULT

1. Subtract the absorbance of blank from all data, including standards and unknown samples before plotting.
2. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (ng/mL)	Absorbance (492nm)
400	2.614
200	1.492
100	0.742
50	0.384
25	0.207
12.5	0.127
6.25	0.080
0	0.034



* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity of the assay is 3ng/mL

2. Specificity

Compound	Cross Reactivity
L-FABP	100.0%
I-FABP	≤ 0.1%

3. Repeatability

The CV Value is no more than 10%, in case of 8 times simultaneously measure of same specimen.

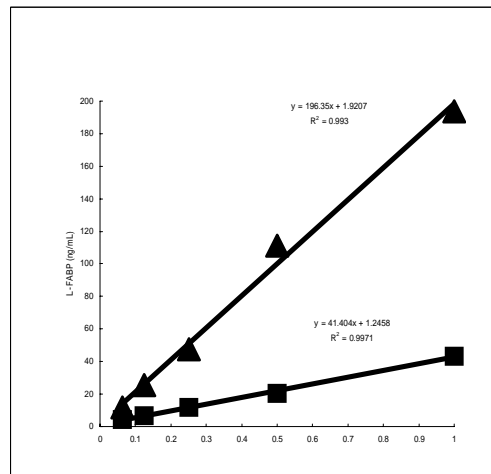
Measurement Value(ng/mL)	S D Value	C V Value(%)	n
240.98	8.53	3.5	8
52.40	4.15	7.9	8
9.85	0.67	6.8	8

The CV Value is no more than 15%, in case of 8 days simultaneously measure of same specimen.

Measurement Value(ng/mL)	S D Value	C V Value(%)	n
227.11	21.87	9.6	8
46.69	5.27	11.3	8
11.02	1.31	11.9	8

4. Dilution test

Specimen	Dilution (X)	Measurement Value(ng/mL)
Human Urine (A)	1	193.85
	2	111.12
	4	47.70
	8	25.55
	16	11.82
Human Urine (B)	1	43.27
	2	20.46
	4	11.96
	8	6.49
	16	4.28



5. Added Recovery Assay

	Additive Concentration (ng/mL)	Measurement Value (ng/mL)	Theoretical Value (ng/mL)	Recovery Rate (%)
Human Urine No.1	0	7.76		
	100	57.33	57.76	99.3
	200	128.54	107.76	119.3
	400	224.71	207.76	108.2
Human Urine No.2	0	17.12		
	100	66.06	67.12	98.4
	200	138.34	117.12	118.1
	400	223.85	217.12	103.1
Human Urine No.3	0	24.30		
	100	69.60	74.30	93.7
	200	134.02	124.30	107.8
	400	260.12	224.30	116.0

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 ~ 8°C. All reagents shall be brought to room temperature approximately 30minutes before use.
2. The measurement result is influenced by time and the temperature of the reaction. Perform all the operations for standards and test samples at the same time under the same condition.
3. Stop Solution is a strong acid substance. Therefore, be careful not to have your skin and clothes contact Stop Solution and pay attention to the disposal of Stop Solution.
4. The Substrate tablets should be transferred to the vessel for reconstitution solution, and avoid direct touch the tablets with hand.
5. Assay Buffer, Standard Diluent and L-FABP Standard contain sodium azide. Therefore, dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE and THE TERM OF VALIDITY

Storage Condition : 2 ~ 8°C

The term of Validity : 12months(The expiry date is specified on outer box.)

PACKAGE UNIT

96 Test

REFERENCE

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