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 (14kDa) expressed in the proximal tubule peculiarly in the kidney, and L-FABP plays important physiological roles in energy and lipid metabolism in proximal tubule which serves function of re-absorption,

L-FABP is useful as a prognosis marker in kidney diseases as it excretes into urine in response to initial symptoms such as stresses caused by the protein urea overload and ischemic stress of micro circulation.

This product is a sandwich type ELISA kit that uses monoclonal antibodies that is able to recognize human type L-FABP in specific, and it enables stable assay result with high sensitivity. Moreover, the L-FABP Antibody Coated Microplate is allowed to be separated for measurement of a small number of specimen materials.

PRINCIPLE

ELISA (Enzyme-Linked-Immuno-Sorbent Assay) of 2-step sandwich method is used for this kit. L-FABP Standard or urine samples are pretreated with Pretreatment Solution, and poured into L-FABP Antibody Coated Microplate on which Assay Buffer is placed and incubated. During this incubation process, L-FABP in the reacting solution binds to the immobilized antibody. As the second antibody, The 2nd Ab-POD Conjugate is added after washing procedure to make L-FABP antigen be sandwiched between immobilized antibody and conjugate antibody. The plate with sandwiched L-FABP antigen is washed and added with Substrate for enzyme reaction process. Changes of color of samples appear according to quantity of L-FABP antigen. Microplate reader records optical density to draw a calibration curve of L-FABP concentration.

MEASUREMENT RANGE

3 ~ 400 ng/mL

INTENDED USE

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 2nd Ab-POD Conjugate	12mL x 1
6.	Substrate	2
7.	Substrate Diluent	12mL x 2
8.	Wash Agent (x 40 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
   - -Plate mixer
   - -Microplate reader: Wave length of 492nm (630nm)
   - -Plate Seal (Attached to each kit)
2. Preparation of wash solution
   Add distilled water to Wash Agent (x 40 concentrate) and prepare 2,000mL of wash solution.
3. Operation method
   Make sure that all reagent reach room temperature approximately 30 minutes prior to use and that no quality changes in all reagent after tilting and mixing gently. Measure diluted L-FABP Standard while measuring test samples to set standard curve.

   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). Take 50uL of the mixed solution from B1well to C1well and mix it gently.
      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.

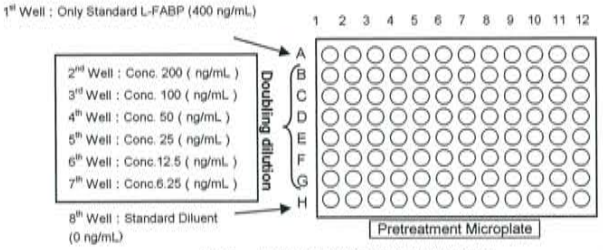

Fig.1 Example of operating pretreatment

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 well.
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 should 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 each well with 350uL of wash solution 3 times.
9) Pipette 100uL of "The 2nd Ab-POD Conjugate" into the wells of test samples, standards involving zero (0) concentration.
10) Seal the plate and stir it for 5 minutes with the plate mixer, and incubate the plate for 55 minutes at room temperature (20 ~ 28°C).
11) Substrate solution: One Substrate tablet is reconstituted in 12mL of "7.Substarate Diluent (1 bottle)" and used as a substrate solution. The substrate solution should be prepared 15 minutes prior to use.
12) After incubation of step 10), remove the liquid, and wash the plate 3 times in the same manner with 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 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. Set microplate reader and read absorbance which is confirmed on the wavelength (Measurement wavelength: 492nm, Background wavelength: 630nm).
17) Plot standard curve based on the absorbance of L-FABP Standard and calculate the amount of L-FABP in the specimen.

Fig.2 Operation Protocol

	Test Sample	Standard	Zero (0) concentration
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 measure absorbance at the wavelengths (Predominant: 492nm, Subdominant: More than 620nm) within 30 minutes after adding of Stop Solution.			

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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 be a cause of failure of measurement.
7. Do not seal solution with Substrate with cap too tight because substrate solution releases bubbles during reaction. Do not reuse the Substrate Solution
8. Measurement should be finished within 30 minutes after adding "Stop Solution".

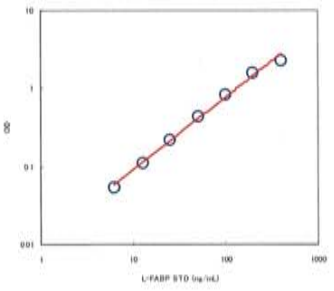
CALCURATION OF TEST RESULT

1. Subtract the absorbance of zero (0) concentration 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-630nm)
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 standard curve above is shown as an example. Set up 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.

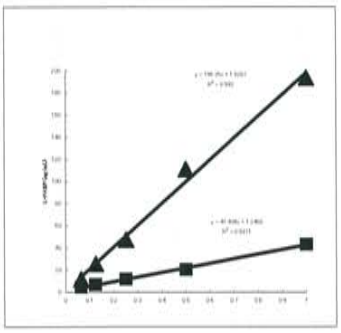
Measurement Value(ng/mL)	SD Value	CV Value(%)	n
240.98	8.53	3.5	8
52.40	4.15	7.9	8
9.85	0.67	6.8	8

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

Measurement Value(ng/mL)	SD Value	CV 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 Vlue(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 should be let warm to room temperature approximately 30minutes prior to use.
2. Measurement result may be influenced by time and temperature of reaction. Perform all measurement of L-FABP standards and test samples under the same conditions.
3. Stop Solution is a strong acid substance. Keep your skin and clothes away from direct contact Stop Solution and pay careful attention when you dispose Stop Solution.
4. Substrate tablets should be transferred to the vessel for reconstitution solution, and avoid touching tablets directly by hand.
5. Sodium azide is contained in Assay Buffer, Standard Diluent and L-FABP Standard.. Dispose these materials after diluting them with large quantity of water to avoid production of explosive metallic azide.
6. Some reagents contains component of animal blood. Handle reagents carefully and wash hands after measurement.
7. Do not mix the reagents with the reagents from different lot or different kind of kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE and VALID TERM

Storage Condition: Store at 2 ~ 8°C

Valid Term: 24 months (Expiry date is printed on kit package and labeling of each kit component.)

PACKAGE UNIT

96 Test

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VERSION

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