Human L-FABP ELISA TMB Kit

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) peculiarly expressed in the proximal tubule 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 is excretes into urine in response to initial symptoms such as stresses caused by the proteinuria 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 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. L-FABP Antibody Coated Microplate is washed following by the L-FABP binding reaction process. 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 again 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

1.5 ~ 200ng/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	12mL x 1
4.	Assay Buffer	12mL x 1
5.	The 2nd Ab-POD Conjugate	12mL x 1
6.	Substrate Solution	12mL x 1
7.	Wash Agent (x 40 concentrate)	50mL x 1
8.	Stop Solution	12mL x 1
9.	Standard Diluent (0ng/mL)	2.5mL x 1
10.	L-FABP Standard (400ng/mL)	0.5mL x 1

OPERATION MANUAL

1. Required Instruments and Equipments
   -Micropipette: Adjustable to 20μL, 50μL
   -Multichannel micropipette: Adjustable to 50μL, 100μL
   -Graduated cylinder: 2,000mL
   -Plate mixer
   -96 well microplate reader: Wave length of 450nm (over 610nm)
   -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. Measuring operation method
   Make sure that all reagents are at room temperature approximately 30 minutes prior to use and tilt and mix each regent itself gently few times to check no quality would change in all reagents. Measure diluted L-FABP Standard while measuring test samples to set standard curve.

(1) Preparation of L-FABP Standards
   1) As shown in Fig.1, use the first column (A1 ~ H1wells) of “2.Pretreatment Microplate” for the preparation.
   2) Add by 50μL of “9.Standard Diluent (0ng/mL)”to each well individually from B1 to H1 in “2.Pretreatment Microplate”.
   3) Add 50μL of “10.L-FABP Standard (400ng/mL)”and 50 μL of “9.Standard Diluent (0ng/mL)” into A1well (Conc. 200 ng/mL), and mix A1well gently (ten times pipetting).
   4) Take 50μL of the mixed solution from A1well and add to B1well and mix them gently.
   5) Continue to perform this doubling dilution procedure from B1well to G1well in the same manner one by one and take out 50μL of the solution from G1well.

(2) Pretreatment
   1) After the preparation of L-FABP Standards, add by 50μL of sample specimens into the other wells individually (A2, B2,…) in “2.Pretreatment Microplate”.
   2) Add by 50μL of “3.Pretreatment Solution” individually to all wells containing L-FABP Standards and the samples specimens. Seal the plate and stir it for more than 5 minutes with a plate mixer.

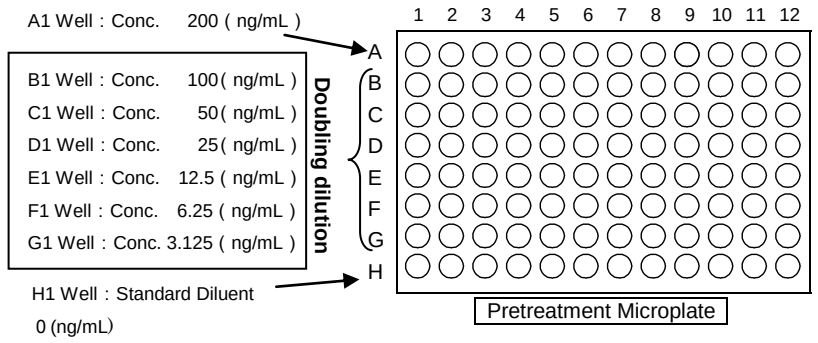

Fig.1 Example of operating pretreatment

(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 by 100μL of “4.Assay Buffer” in each well.

(4) Pipette the standard solution from each well in the first column in “2. Pretreatment Microplate” and add the standard solution (20μL/well) to respective two wells in the first two strips in“1. L-FABP Antibody Coated Microplate”.

(5) Pipette by 20μL 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 a plate mixer, and then incubate “1. L-FABP Antibody Coated Microplate” for 55 minutes at room temperature(20 ~ 28℃).

(7) After the incubation, throw away the liquid from “1.L-FABP Antibody Coated Microplate”.

(8) Wash each well in “1. L-FABP Antibody Coated Microplate” with wash solution (350μL/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 350μL of wash solution 3 times.

(9) Pipette by 100μL of “5.The 2nd Ab-POD Conjugate” into the wells of test samples, standards involving zero concentration.

(10) Seal the plate and stir it for 5 minutes with a plate mixer, and incubate the plate for 25 minutes at room temperature (20 ~ 28℃).

(11) After incubation of step (10), remove the liquid, and wash the plate 3 times in the same manner as step (8).

(12) Pipette by 100μL of “6.Substrate Solution” into the wells.

(13) Seal the plate and stir it for 5 minutes with a plate mixer, and incubate the plate for 25 minutes at room temperature (20 ~ 28℃) in the dark.

(14) Pipette 100μL of “8.Stop Solution” into the wells. Mix the liquid by tapping the side of the plate.

(15) Remove any dirt or drop of water on the bottom of the plate and check there is no bubble on the surface of the liquid. Set 96 well microplate reader and read absorbance which is confirmed with the wavelength (Dominant wavelength: 450nm, Secondary wavelength: over 610nm).

(16) Plot standard curve based on the absorbance of “10.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 50μL	L-FABP Standard 50μL	Standard Diluent (0ng/mL)50μL
	Pretreatment reagent 50μL		
Mix for more than 5 minutes by Plate Mixer after sealing plate			
Assay Buffer	100μL	100μL	100μL
Pretreated samples	20μL	20μL	20μL
Mix for 5 minutes by Plate Mixer after sealing plate			
Incubate for 55 minutes at room temperature			
Wash 3 times			
Labeled antibody	100μL	100μL	100μL
Mix for 5 minutes by Plate mixer and after sealing plate			
Incubate for 25 minutes at room temperature			
Wash 3 times			
Substrate Solution	100μL	100μL	100μL
Mix for 5 minutes by Plate mixer and after sealing plate			
Incubate for 25 minutes at room temperature with light shielding			
Stop Solution	100μL	100μL	100μL
Tap the plate for mixing and measure absorbance at the wavelengths (Dominant wavelength: 450nm, Secondary wavelength: over 610nm) within 30 minutes after adding of Stop Solution.			

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SPECIAL ATTENTION

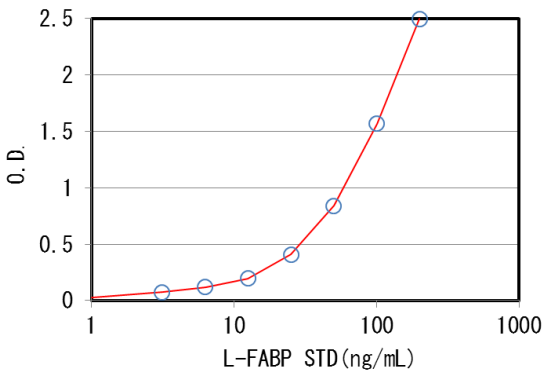
1. Test samples should be measured soon after collection. For storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before the measurement.
2. Test samples should be diluted with Standard Diluent as needed basis.
3. Put an unused L-FABP Antibody Coated Microplate in a bag and preserve it in refrigerator until when it will be used for the next time.
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 completed within 30 minutes after adding Stop Solution.

CALCURATION OF TEST RESULT

1. Subtract the absorbance of zero concentration from all data, including standards and unknown samples before plotting to calculate specific Optical Density (Net O.D.) of respective wells.
2. Plot the Net O.D. of L-FABP standard in vertical axis and L-FABP concentration in horizontal axis 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

L-FABP Conc. (ng/mL)	O.D. (450nm)
200	2.497
100	1.481
50	0.842
25	0.423
12.5	0.226
6.25	0.122
3.125	0.072
0 (Blank)	0.019



* The standard curve above is shown as an example. Set up standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Sensitivity

   The minimal sensitivity of the assay is 1.5 ng/mL.

2. Specificity

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

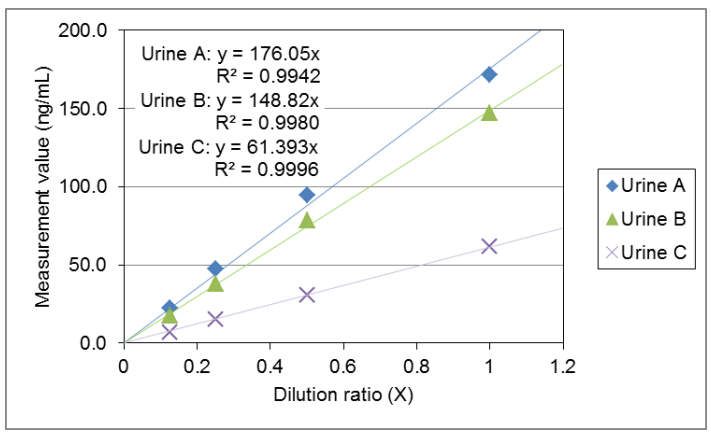
3. Repeatability

   The CV value is not more than 15%, in case of 8 times simultaneously measurement of the same specimen.

Measurement value(ng/mL)	SD	CV(%)	n
113.0	3.7	3.3	8
50.3	6.6	13.1	8
16.3	0.6	3.7	8

4. Dilution test

Sample	Dilution ratio (×)		Measurement value(ng/mL)
Urine A	1/1	1.0	171.8
	1/2	0.5	94.6
	1/4	0.25	47.6
	1/8	0.125	22.5
Urine B	1/1	1.0	146.9
	1/2	0.5	78.2
	1/4	0.25	37.9
	1/8	0.125	17.4
Urine C	1/1	1.0	61.7
	1/2	0.5	30.4
	1/4	0.25	15.0
	1/8	0.125	7.1



PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 ~ 8℃. All reagents should be brought back to room temperature approximately 30minutes prior to use.
2. Measurement result may be influenced by the time and the 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 Stop Solution and pay careful attention when you dispose.
4. Assay Buffer, Standard Diluent and L-FABP Standard contain Sodium azide. Make sure diluting them with large quantity of water before dispose these materials to avoid production of explosive metallic azide.
5. Some reagents contains component of animal blood. Handle reagents carefully and wash hands after the measurement.
6. Do not mix the reagents with the reagents from different lot or different kind of kit.
7. Do not use expired reagents.
8. This kit is produced for research purpose only. Do not use for clinical diagnosis.

STORAGE and VALID TERM

Storage Condition: Store at 2 ~ 8℃
Valid Term: 24 months (Expiry date is printed on the kit package and labeled on each component.)

PACKAGE UNIT

96 Tests

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VERSION

October 2015 Established.
November 2015 Revised. (Version 2)

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