Instructions For Use

Revised in June 2017

RENISCHEM® L-FABP ELISA High Sensitivity Kit

A kit for the quantitative determination of human L-FABP in urine

IVD

For in vitro diagnostic use only.

CE

INTENDED USE

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

It is intended to subserve diagnosis of renal disease accompanied by renal tubule dysfunction.

INTENDED USER

Lab Professional

KIT COMPONENT

Reagents

SYMBOL	Name	Quantity	Chief ingredients
PLT	L-FABP Antibody Coated Microplate	96 Well x 1	96-well strip plate coated with anti-human L-FABP mouse monoclonal antibody (CloneL)
P	Pretreatment Solution	12 mL x 1	Buffering agent, Surfactant
B	Assay Buffer	12 mL x 1	Buffering agent
C	The 2nd Ab-POD Conjugate	12 mL x 1	Anti-human L-FABP mouse monoclonal antibody (Clone2) conjugated to horseradish peroxidase (0.1 – 1.0μg/mL)
SBS	Substrate Solution	12 mL x 1	TMB (3, 3', 5, 5'-tetramethylbenzidine), hydrogen peroxide
W	Wash Agent (x40 concentrate)	50 mL x 1	Buffering agent, Surfactant
S	Stop Solution	12 mL x 1	1N Sulfuric acid
STDD	Standard Diluent (0ng/mL)	2.5 mL x 1	Buffering agent
STD	L-FABP Standard (400ng/mL)	0.5 mL x 1	Recombinant human L-FABP, Buffering agent

PPLT Pretreatment Microplate : 96 Well x 1

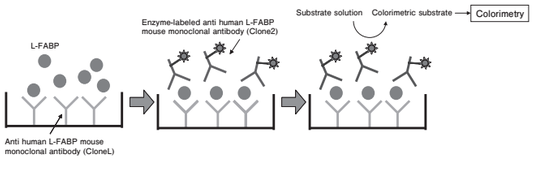
PS Plate Seal : 2 sheets

PRINCIPLE

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

L-FABP Standard and urine samples are firstly treated with Pretreatment Solution which enhances the antigen recognition and reactivity of anti L-FABP antibody, 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.



WARNINGS AND PRECAUTIONS

General Precautions

1. User should read these Instructions For Use carefully.
2. Any off-label use of this product, deviation from the instructions given in this manual, and/or modification of the components avoid the manufacturer's liability.
3. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect assay result.
4. Take into account other assay results and clinical symptoms in conjunction with the measurement result of this kit in order to diagnose comprehensively.
5. User should also read Instructions For Use of instruments and equipment required in combination with this product.

Safety Issues

Some reagents in this kit contain hazardous substances as follows.

1. Hazard identification: Sodium azide

Assay Buffer, Standard Diluent (0ng/mL) and L-FABP Standard (400 ng/mL) contain sodium azide as a preservative. (0.1% w/v, 0.5% w/v and 0.5% w/v, respectively)

SIGNAL WORD: Danger

HAZARD STATEMENTS:

H300 : Fatal if swallowed.
H400 : Very toxic to aquatic life.
H410 : Very toxic to aquatic life with long lasting effects.

PRECAUTIONARY STATEMENTS:

P264 : Wash hands thoroughly after handling.
P273 : Avoid release to the environment.
P301+P310 : IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician.
P330 : Rinse mouth
P501 : Dispose of contents/container to the waste disposer authorized by prefectural governor.

SUPPLEMENTAL HAZARD INFORMATION

EUH032 : Contact with acids liberates very toxic gas.

2. Hazard identification: Sulfuric acid

Stop Solution contains Sulfuric acid (4.9% w/v).

SIGNAL WORD: Warning

HAZARD STATEMENTS:

H315 : Causes skin irritation.
H319 : Causes serious eye irritation.

PRECAUTIONARY STATEMENTS:

P280 : Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352 : IF ON SKIN: Wash with plenty of soap and water.
P332+P313 : If skin irritation occurs: Get medical advice/attention.
P362 : Take off contaminated clothing and wash before reuse.
P305+P351+P338 : IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313 : If eye irritation persists: Get medical advice/attention.

SUPPLEMENTAL HAZARD INFORMATION: Not applicable

Handling Cautions

1. Wear disposable protective gloves while handling reagents and specimen. Do not pipette by mouth.
2. Some reagents contain a substance of animal origin. Wash hands thoroughly afterwards.

Operating Precautions

1. Do not use or mix with reagents from different lot or kit.
2. Only use Wash Agent contained in the kit for washing L-FABP Antibody Coated Microplate. Insufficient washing may lead to failure in measurement.
3. Cover the plate with a plate seal tightly during reaction. Higher concentration of reaction solution caused by evaporation may result in higher absorbance value.
4. Confirm there is no dirt on the bottom of the plate before measuring absorbance values. The measurement should be conducted as quickly as possible (within 30 minutes), because it is confirmed that the absorbance value will decrease after the addition of Stop Solution.
5. The measurement result will be influenced by time and the temperature at reaction. Perform all operations for standard and test sample at the same time under the same condition.
6. Draw a standard curve for each assay.

Disposal Cautions

1. Dispose of reagents and accessories in compliance with local authority's requirements.
2. 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.
3. Stop Solution is an acid substance. Therefore, pay extra attention to in disposal of this material.
4. Specimen should be considered potentially infectious. Dispose of used instruments (e.g. pipettes, tubes), waste solution and sampling tips after decontamination by one of the following methods:
   · Soak in 0.05% of formalin solution at 37°C for 72 hours or more.
   · Soak in 2% of glutaraldehyde solution for 1 hour or more.
   · Soak in sodium hypochlorite solution (1,000 ppm effective chlorine concentration) for 1 hour or more.
   · Autoclave at a temperature of 121°C for 20 minutes.

STORAGE AND STABILITY

1. Store at 2 - 8°C and bring to 20 - 28°C before use. Do not freeze.
2. Performance of the kit is assured until its expiry date printed on the kit label under the following conditions, it is unopened and it is stored in a proper condition.
3. Do not use reagents that have been expired or frozen.
4. Once opening, performance of the kit is stable for 30 days under being stored at 2 - 8°C.
5. Do not store nor reuse wash agent after preparation.
6. In case of storing an unused portion of L-FABP Antibody Coated Microplate, put it in a bag with a desiccant and zip the bag. Then seal the bag tightly with tape and preserve it at 2 - 8°C until next use.

SPECIMEN COLLECTION

1. Test sample should be measured soon after collection. In case of storing test sample, store those frozen (- 20 - - 80°C) and do not repeat freeze/thaw cycles. Thaw the test sample by leaving them at room temperature or leaving them at 2 - 28°C in water bass and mix them completely before measurement. It is confirmed that test sample frozen-stored at - 80°C is stable for 1 year.
2. Test sample should be diluted with Standard Diluent if required. If the measurement result exceeds 60ng/mL, dilute and measure again.
3. Use test sample in neutral pH range. The contaminations of organic solvent may affect the measurement.

INTERFERING SUBSTANCES

1. Higher levels of bilirubin, hemoglobin, glucose or ascorbic acid in urine specimen than the following ranges may interfere with assay performance.
   · Free bilirubin in urine up to 19.7 mg/dL does not affect the measurement value.
   · Conjugated bilirubin in urine up to 21.8mg/dL does not affect the measurement value.
   · Hemoglobin in urine up to 24.4 mg/dL does not affect the measurement value.
   · Glucose in urine up to 45mg/dL does not affect the measurement value.
   · Ascorbic acid in urine up to 12.5 mg/dL does not affect the measurement value.
2. Measurement within 24 hours after the administration of angiographic contrast agent may lead to a higher value of L-FABP in urine due to a transient renal ischemia.

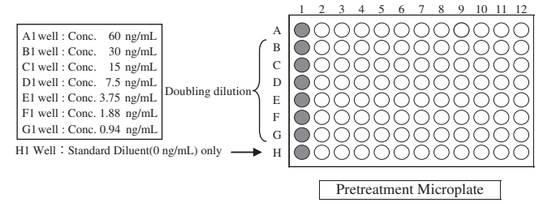
TEST PROCEDURE

Instruments and Equipment Required

1. Micropipette: adjustable to 15 - 85 μL
2. Multichannel micropipette: adjustable to 50 μL, 100 μL
3. Graduated cylinder: 2,000 mL
4. Plate washer or a washing jar
5. Plate mixer
6. Microplate reader: wavelength range 450 nm (reference wavelength 610 nm or over)

Preparation

1. Substrate solution : Use TMB (3, 3', 5, 5'-tetramethylbenzidine) substrate solution directly.
2. Wash solution : Dilute a half volume (25mL) of Wash Agent (x40 concentrate) with distilled water to make 1,000 mL of wash solution. Prepare the wash solution for each test run. Do not store nor reuse the wash solution.
3. Preparation of L-FABP Standard solution:
   1) Use the first column ("A1 - H1" wells) of Pretreatment Microplate for the preparation.
   2) Add 50 μL of Standard Diluent (0ng/mL) to the "B1, C1, D1, E1, F1, G1 and H1" wells of the Pretreatment Microplate.
   3) Add 15μL of L-FABP Standard (400ng/mL) and 85μL of Standard Diluent (0ng/mL) to the "A1" well, and mix them gently (by 10 times pipetting). Then take 50 μL of this solution and add to the "B1" well. Mix it as well.
   4) Take 50μL from the "B1" well and add to the "C1" well in the same manner as the above.
   5) Complete the steps of double dilution as taking and discarding 50 μL from the "G1" well after mixing.
   6) Set the "H1" well as "blank" with 50 μL of Standard Diluent (0ng/mL) only.



Operation

The kit and all reagents shall be brought to 20 - 28°C before use. All reagents shall be mixed gently and completely, and make sure there is no change in quality of the reagents before use. Prepare a standard curve while simultaneously measuring test sample and L-FABP Standard solution.

1. Pretreatment :
   1) After preparation of L-FABP Standard solution, add 50μL of sample specimen to the wells from the second column ("A2, B2,…") of the Pretreatment Microplate.
   2) Add 50μL of Pretreatment Solution to each well added with the L-FABP Standard solution or the sample specimen.
   3) Cover the plate with a plate seal and stir it for more than 5 minutes with a plate mixer.
2. Set the strips of L-FABP Antibody Coated Microplate (a strip for the L-FABP Standard solution + strips for the sample specimen) in the plate holder. Add 100μL of Assay Buffer to each well.
3. Transfer 20μL of the pretreated L-FABP Standard solution from the Pretreatment Microplate to the L-FABP Antibody Coated Microplate.
4. Transfer 20μL of the pretreated sample specimen from the Pretreatment Microplate to the L-FABP Antibody Coated Microplate.
5. Cover the L-FABP Antibody Coated Microplate with a plate seal and stir it for 5 minutes with a plate mixer. Then incubate it for 55 minutes at 20 - 28°C.
6. After the incubation, remove the reaction solution from the L-FABP Antibody Coated Microplate.
7. Add 350μL of the Wash solution to each well and remove the Wash solution. Repeat this washing procedure for 3 times. Then remove the remaining liquid from all wells by blotting on paper towels. (In case of using a plate washer, however, wash 3 times with 350μL of the Wash solution.)
8. Add 100μL of The 2nd Ab-POD Conjugate to each well.
9. Cover the plate with a Plate Seal and stir it for 5 minutes with a plate mixer. Then incubate it for 25 minutes at 20 - 28°C.
10. After the incubation, remove the reaction solution from the wells. Wash the plate for 3 times in the same manner as step 7.
11. After removing the remaining liquid from all wells, add 100μL of Substrate Solution to the wells.
12. Cover the plate with a Plate Seal and stir it for 5 minutes with a plate mixer under light shielding. Then incubate it for 25 minutes at 20 - 28°C under light shielding.
13. Add 100μL of Stop Solution to the wells to terminate the enzyme reaction.
14. Mix the liquid by tapping the side of the plate. Within 30 minutes, read the absorbance of each well at 450nm using a microplate reader. If a dual wavelength plate reader is available, set the test wavelength at 450nm and reference at 610nm or over.
15. Draw a standard curve based on the absorbance of L-FABP Standard solution, and determines the L-FABP quantity in the sample specimen.

Operation Protocol

	Test sample	Standard	Blank
Pretreatment	Test sample 50µL	L-FABP Standard 50µL	Standard Diluent (0ng/mL) 50µL
	Pretreatment solution 50µL		
Mix for more than 5 minutes with a plate mixer after sealing the plate.			
Assay Buffer	100µL	100µL	100µL
Pretreated samples	20µL	20µL	20µL
Mix for 5 minutes with a plate mixer after sealing the plate.			
Incubate for 55 minutes at 20 - 28°C.			
Wash 3 times.			
The 2nd Ab-POD Conjugate	100µL	100µL	100µL
Mix for 5 minutes with a plate mixer after sealing the plate.			
Incubate for 25 minutes at 20 - 28°C.			
Wash 3 times.			
Substrate solution	100µL	100µL	100µL
Mix for 5 minutes with a plate mixer after sealing the plate.			
Incubate for 25 minutes at 20 - 28°C under light shielding.			
Stop Solution	100µL	100µL	100µL
Tap the plate for mixing. Measure the absorbance at 450nm (reference at 610nm or over) within 30 minutes after the addition of Stop Solution.			

CALCULATION OF TEST RESULT

1. Subtract the absorbance of the Standard Diluent (0ng/mL) from absorbance of each well for adjustment.
2. Plot the adjusted absorbance of L-FABP Standard solution against the concentration of L-FABP Standard solution to draw a standard curve.
3. Determine the L-FABP concentration in the sample specimen by applying the adjusted absorbance of sample specimen to the standard curve.
4. Calculate the L-FABP quantity (µg/gCr) per 1g of urine creatinine with a urinary creatinine correction.

Reference Intervals

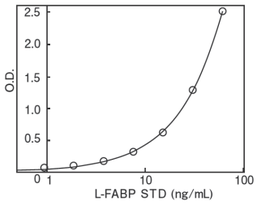
Reference interval calculated from urinary L-FABP quantity of 412 healthy subjects was 8.4µg/gCr or less.

Limitations

Sample specimen that has L-FABP concentration value greater than 60ng/mL should be diluted with Standard Diluent (0ng/mL) and re-assayed.

Example of measured values and standard curve

L-FABP concentration (ng/mL)	Absorbance (450 nm)
60	2.508
30	1.278
15	0.618
7.5	0.316
3.75	0.170
1.88	0.101
0.94	0.066
0 (blank)	0.031



This figure is made for reference.
Standard curve should be drawn at each assay.

QUALITY CONTROL

In order to confirm that the test functions properly and to demonstrate that the results are valid, the following three conditions must be fulfilled:

1. the absorbance of the Standard Diluent (0 ng/mL) should be 0.1 or less,
2. the absorbance level of the L-FABP Standard (60 ng/mL) should be 1.5 or more,
3. the absorbance values should be monotonously increasing with increase of L-FABP concentration and the concentration of each standard solution determined from the standard curve should not deviate more than 20% from the actual concentration of the standard solution.

CLINICAL IMPLICATIONS

L-FABP is a low molecular soluble protein (about 14kDa) expressed in the proximal renal tubule peculiarly in the kidney, and physiologically it is thought that L-FABP plays an important role in energy and lipid metabolism in the renal tubule where the function of re-absorption of the kidney is borne[1]. L-FABP is induced and excreted into urine in response to stress such as urinary protein and blood micro circulation disorder that may cause renal disease progression[2,13]. Most of conventional renal function markers indicate only the outcomes of renal dysfunction, whereas L-FABP can be used to monitor the degree of those progression[3-5]. Currently, it is considered that life prognosis of patients with diabetes which is the leading cause for dialysis is extremely poor. Measured urinary L-FABP values of those diabetic patients are expected to be useful as a marker for early diagnosis and prevention of diabetic nephropathy progression.

We conducted clinical performance tests on both healthy individuals and diabetic patients. The distribution of their urinary L-FABP values is shown in Figure 1. Urinary L-FABP (µg/gCr) of diabetic nephropathy patients showed significantly higher values than those of healthy individuals. The sensitivity and specificity of L-FABP and type IV collagen which is conventionally used for early diagnosis of diabetic nephropathy are shown in Table 1.

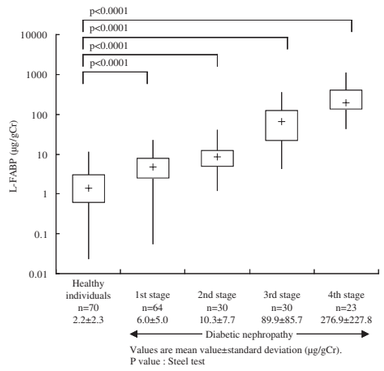


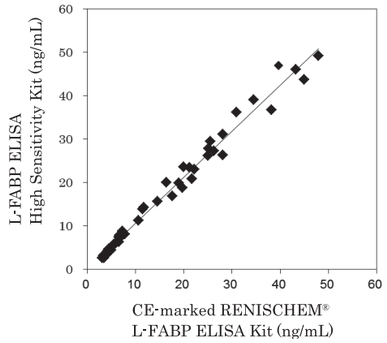
Figure1. Distribution of urinary L-FABP values of healthy individuals and diabetic nephropathy patients in each stage

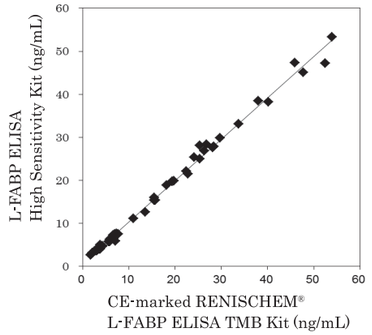
Table 1. Sensitivity and Specificity : Comparison with the conventional diagnostic method

	Sensitivity (diabetic nephropathy patients in each stage)				Specificity
	1st stage	2nd stage	3rd stage	4th stage	Healthy individuals
L-FABP	21.9%	50.0%	96.7%	100.0%	97.1%
Type Ⅳ collagen	9.4%	46.7%	60.0%	91.3%	95.8%

PERFORMANCE CHARACTERISTICS

1. Sensitivity
   The minimum detectable sensitivity of the kit is 0.3 ng/mL under the following conditions;
   1) the absorbance of the Standard Diluent (0 ng/mL) should be 0.1 or less, and,
   2) the absorbance of the L-FABP Standard (60 ng/mL) should be 1.5 or more.
2. Accuracy
   When measuring control reagents for two different kinds of known concentrations (0.94-60ng/mL), the each value is in the concentration range that is ±20% of the known concentration.
3. Repeatability
   When measuring the same specimen for two or more kinds of concentrations (0.94-60ng/mL), for 8 times simultaneously, the each CV value is 15 % or below 15%.
4. Substance
   (1) Control reagent for accuracy test
   Standard L-FABP is defined by calibrator which consists of Human L-FABP recombinant protein derived from microorganism. Control reagent of known concentration is L-FABP Standard solution which is made from diluted L-FABP Standard.
   (2) Control sample for repeatability test
   Control sample is made from human urine samples which are pre-adjusted with human urine sample in order to be the defined L-FABP concentration.
5. Measurement range
   When testing with SPECTRA Max 340PC^{384} Microplate Spectrophotometer by Molecular Devices, the measurement range is 0.3 – 60 ng/mL.
6. Correlative evaluation
   The following figures show the result of correlative evaluation between RENISCHEM® L-FABP ELISA High Sensitivity Kit and the two CE-marked in-vitro diagnostic products.
   (Two CE-marked in-vitro diagnostic products : 1) RENISCHEM® L-FABP ELISA Kit 2) RENISCHEM® L-FABP ELISA TMB Kit)





7. Reference Material for Calibration
   Human L-FABP recombinant protein

PACKAGE UNIT

96 Tests

TRACEABILITY

Following ISO17511:2003, the calibration of the urinary L-FABP assay is traceable to the manufacturer's standing measurement procedure using the manufacturer's product calibrator (recombinant human L-FABP).

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■GLOSSARY OF SYMBOLS

IVD	In Vitro Diagnostic Medical Device	SBS	Substrate Solution
CE	CE Marking (European directive 98/79/EC on in vitro diagnostic medical device.)	REF	Catalogue number
	Consult Instructions for Use	LOT	Batch code
	Temperature limitation		Used by
	DO NOT REUSE		Manufacturer
	Danger	EC REP	Authorized representative in the European Community
	Warning	W	Wash Agent (x40 concentrate)
	Warning	S	Stop Solution
PLT	L-FABP Antibody Coated Microplate	STDD	Standard Diluent(0ng/mL)
P	Pretreatment Solution	STD	L-FABP Standard(400ng/mL)
B	Assay Buffer	PPLT	Pretreatment Microplate
C	The 2nd Ab-POD Conjugate	PS	Plate Seal
		CONTENTS	Contents of kits

CMIC HOLDINGS Co.,Ltd.
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EC REP
Emergo Europe
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