Instructions For Use

Revised in June 2017

RENISCHEM® L-FABP POC Kit

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

REF KZ-002

IVD

For in vitro diagnostic use only

CE

INTENDED USE

RENISCHEM® L-FABP POC Kit is capable of the detection and semi-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 Professionals

KIT COMPONENTS

SYMBOL	Name	Quantity	Chief ingredients
TC	L-FABP Test Cassette	x 1	Anti-human L-FABP mouse monoclonal antibody (Clone2), Gold colloid anti-human L-FABP mouse monoclonal antibody (CloneL)
PT	Pre-treatment Micro Tube	x 1	Pre-treatment reagent (blue-colored reagent)
DP	Dropper	x 1	Disposable plastic pipette
-	Dry sheet	x 1	Drying reagent

All kit components are pre-packed into aluminum pouch (10 units/box) which each contains the following components in sufficient quantities for 10 individual determinations.

- 10 individual test kits
- 10 reference cards
- 1 Instructions For Use

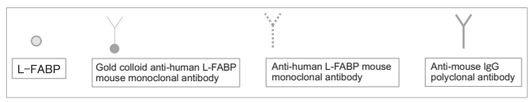
SUMMARY AND EXPLANATION OF THE TEST

L-FABP is expressed as a low-molecular-weight soluble protein of approximately 14 kDa in a proximal tubule, which is responsible for reabsorption function in a kidney, playing an important role in metabolism of energy and lipid[1, 3]. L-FABP is excreted to the urine in response to proteinuria observed by the progression of kidney disease or ischemic and oxidative stresses [2, 9]. L-FABP can be detected in the early stage of the progression of kidney disease, although most of biomarkers reflect the result of failure of kidney function [4, 5]. Many of clinical trials using the CE-marked product "RENISCHEM®" report that excretion levels of L-FABP into urine can reflect the degree of progression of diabetic nephropathy and L-FABP, the diagnostic index is a highly sensitive biomarker for kidney disease[14,15,21].

PRINCIPLE

RENISCHEM® L-FABP POC Kit is an immunochromatographic test for **detection and semi-quantitative determination of L-FABP. Urinary L-FABP levels can be visualized within 15 minutes after the specimen is added to the L-FABP Test Cassette.** L-FABP in the urine samples reacts to a gold colloid anti-human L-FABP mouse monoclonal antibody (CloneL), forms an antigen-antibody complex, move through the membrane by a capillarity action, and reacts to the anti-human L-FABP mouse monoclonal antibody on the Test Line (Clone2), visualizing a burgundy color. RENISCHEM® L-FABP POC Kit allows semi-quantitative measurement by comparing the color intensity of Test Line with that of reference card.

Additionally, the gold colloid anti-human L-FABP mouse monoclonal antibody (CloneL) moves further on the membrane and binds with the anti-mouse IgG polyclonal antibody on the Control Line, visualizing a burgundy color. This line is an index for checking that the test is performed properly.



WARNINGS AND PRECAUTIONS

General Precautions

1. The user should read Instructions For Use and keep it if there is a need.
2. This kit is intended to be used for in vitro diagnostic use only. Do not use for any other purpose.
3. Comprehensive diagnosis should be made results of lab tests, consideration of clinical symptoms, etc., in addition to the result of the measurement by this kit.
4. Components stored under conditions over 30°C or below 1°C may not perform properly and may adversely affect the assay result.
5. Do not interchange reagents or L-FABP Test Cassette from any different production lots.

Handling Cautions

1. Wear disposable protective gloves while handling reagents and specimen. Do not pipette by mouth.
2. Some reagents contain a substance derived from animals. Wash hands thoroughly after measurement procedures.
3. If reagent gets into eyes or mouth, take necessary emergency procedures, such as washing hands with water, and seek doctor's treatment as needed.
4. Take care to avoid injury with aluminum pouch when opening.
5. This product may jump out of the aluminum pouch if it is pulled too hard when opening.

Operating Precautions

1. Follow the operation method described Instructions For Use when using the product.
2. Use this product at 10-25°C.
3. Be sure to use the reference card enclosed in the kit for result assessment.
4. Do not mix/replace any components, including reference card, with/from that of other kits or any kits from different production lots.
5. Use L-FABP Test Cassette as quickly as possible after opening aluminum pouch.
6. Handle this product and specimen under the conditions described in Instructions For Use as measurement result may be affected by reaction time and temperature.
7. No visible line on the Control Line indicate specimen failed to be reacted correctly. In the case, confirm operation method and perform a retest.

Disposal Cautions

1. Dispose of reagents and accessories in compliance with local authorities requirements.
2. Specimen should be considered potentially infectious. Dispose of used instruments (e.g. pipettes, tubes), waste solution and sampling tips after decontamination according to 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 (1,000ppm effective chlorine concentration) for 1 hour or more.
   · Autoclave at a temperature of 121°C for 20 minutes.

STORAGE AND STABILITY

1. Store this kit at 1 - 30°C. Do not freeze.
2. Performance of the kit is quality assured until the expiry date shown on the label, provided that it is unopened and stored under correct conditions.
3. Do not use reagents which are already expired or stored in frozen condition.

SPECIMEN COLLECTION

1. Urine should be collected in a clean and dry sample container.
2. Measure the specimen immediately after urine collection. When measuring the specimen that has been stored, measure it within two days after being put in cold storage or when the specimen is frozen (- 80 to - 20°C), do not repeat freezing and thawing the specimen. In addition, when the specimen frozen is to be thawed, leave it under room temperature or in a water bath (2 to 28°C), and mix it sufficiently prior to measurement. Specimen frozen at -80°C is verified to be stable for 1 year.
3. Apply the specimen mixed with the Pre-treatment reagent immediately to the L-FABP Test Cassette.
4. If the specimen has a foreign body, high viscosity, or deep coloration, the result of assessment may not be accurate.
5. Mix the specimen with the Pre-treatment reagent slowly to avoid bubbling as much as possible. In the case that the mixture with the Pre-treatment reagent shows excessive bubbling, the amount of specimen may be insufficient.
6. Urine collection with added hydrochloric acid may affect measurement results. Urine collection with added toluene does not affect measurement results.

INTERFERING SUBSTANCES

1. Levels of bilirubin, hemoglobin, glucose or ascorbic acid in urine specimen in the following ranges can interfere with assay performance. However the following levels can be present in the urine with no effect on assay value:
   ·Free bilirubin up to 19.7 mg/dL
   ·Conjugated bilirubin up to 21.8 mg/dL
   ·Hemoglobin up to 97.4 mg/dL
   ·Glucose up to 1000 mg/dL
   ·Ascorbic acid in urine up to 1.6 mg/mL
2. Acid urine below pH 5 may affect measurement result.
3. Measurement within 24 hours after the administration of angiographic contrast reagent may lead to a higher value of L-FABP in urine due to a transient renal ischemia.

TEST PROCEDURE

Instruments and Equipment Required

Multichannel micropipette: adjustable to 100µL.

Preparation

1. Open aluminum pouch and place L-FABP Test Cassette on a flat table.
2. Check that the cap of the Pre-treatment Micro Tube containing Pre-treatment reagent (blue solid) is closed tightly.
3. Check that the Pre-treatment reagent is at the bottom of the tube. When the Pre-treatment reagent is not at the bottom of the tube, hold the upper part of the tube and shake lightly down to move the Pre-treatment reagent to the bottom of the tube.
4. Prepare urine sample stored in collection cup.

Operation

1. Take 100µL of urine sample (fill to the line of the Dropper) and add to Pre-treatment Micro Tube (See Illustration 1).
2. Mix the sample with the Pre-treatment reagent slowly 10 times by using Dropper to avoid bubbling as much as possible (See Illustration 2).
3. Take 100µL of the mixed sample (fill to the line of the Dropper). Place all the mixed sample (100µL) into L-FABP Test Cassette (See Illustration 3).
4. Keep L-FABP Test Cassette flat and stable. After 15 minutes, read Test Line of L-FABP Test Cassette. The L-FABP concentration range of the sample is determined.
5. Do not read Test Line shown on L-FABP Test Cassette which has been left for more than 15 minutes following addition of sample.

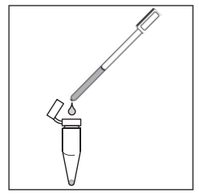


Illustration 1

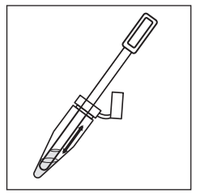


Illustration 2

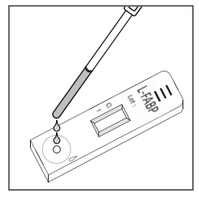
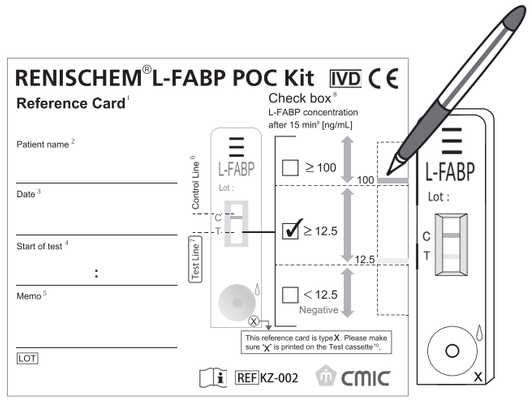


Illustration 3

ASSESSMENT PROCEDURE

The L-FABP concentration range is determined **by comparing the color intensity of the Test Line with the color blocks of the reference card** (See Illustration 4). It is necessary to use the reference card supplied with the kit. Comparing to color density printed on the reference card, tick appropriate box of the L-FABP concentration level which is close to the color density displayed on the Test Line of the L-FABP Test Cassette.



1	Reference Card	6	Control Line
2	Patient name	7	Test Line
3	Date	8	Check box
4	Start of test	9	L-FABP concentration after 15 min [ng/mL]
5	Memo	10	This reference card is type X. Please make sure "X" is printed on the Test cassette.

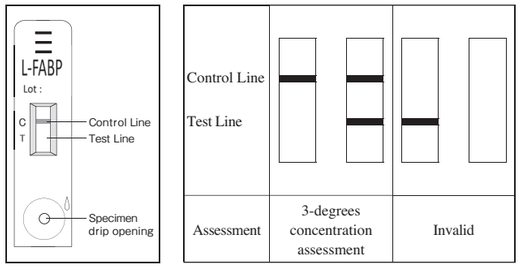
Illustration 4

Documentation and archiving

To document the test result, the concentration range, which corresponds to the color intensity of the Test Line, is marked with a cross on the reference card. To archive the test result, the fully completed reference card can be stuck in the patient file.

TEST RESULT

When a line appears on the Control Line, read the color intensity of the Test Line and make an assessment based on the three degrees of concentration.



Result Score

If both Control Line and Test Line appear in the viewing area, the test indicates that L-FABP was detected in the specimen.

A. 0-12.5ng/mL (L-FABP < 12.5ng/mL): The burgundy color intensity of the Test Line is less than 12.5 of the color block of the reference card.

B. 12.5-100ng/mL (L-FABP ≥ 12.5ng/mL and < 100ng/mL): The burgundy color intensity of the Test Line is more than 12.5 of the color block of the reference card and less than 100.

C. ≥ 100ng/mL (L-FABP ≥ 100ng/mL): The burgundy color intensity of the Test Line is more than 100 of the color block of the reference card.

Invalid

If there is no visible line on the Control Line area within 15 minutes, repeat the assay with a new test device.

QUALITY CONTROL

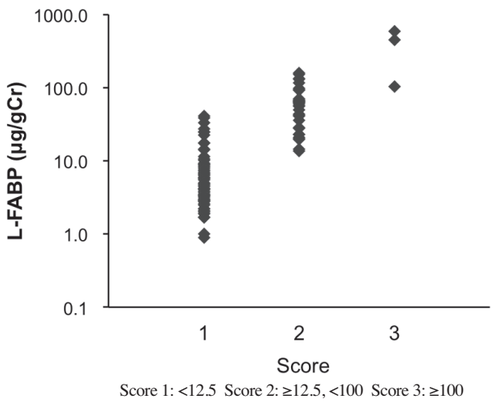
L-FABP Test Cassette contains a built in control feature. The appearance of the burgundy Control Line indicates that the test has been performed correctly and adequately.

CLINICAL IMPLICATIONS

1. Background of development and usefulness in clinical diagnosis
   CMIC HOLDINGS Co.,Ltd. developed the CE-marked product "RENISCHEM®" and the CE-marked product "RENISCHEM® L-FABP TMB Kit" as the world-first in vitro diagnostics that are based on enzyme-linked immunosorbent assay (ELISA) method. Moreover, the usefulness of urinary L-FABP has also been recently indicated in the international guideline[18] and review papers[8,12,13] in acute kidney injury (contrast medium-induced nephropathy[7,20,22,24], kidney transplantation[9], hematopoietic stem cell transplantation[23], cardiac surgery[10,17], sepsis[11], the area of intensive care including newborns[6,16,19,25]), and the establishment of POC (point of care) assay that swiftly estimates prognosis of cardio-renal syndrome and the area of intensive care is expected. On these backgrounds, we developed RENISCHEM® L-FABP POC Kit, which is based on immunochromatographic method. This product can measure urinary L-FABP easily at bed side and gives a negative result under the reference values determined by the clinical trial data for "RENISCHEM®," which has already been approved result score makes it possible to diagnose acute kidney injury swiftly or to estimate the degree of severity[26].
2. Range of reference value
   The range of reference value, which are determined by the distribution of urinary L-FABP corrected by urinary creatinine levels in 431 healthy adults in the clinical performance trial of the CE-marked product, "RENISCHEM®," is 8.4 μg/gCr or below. The range of reference value of urinary L-FABP concentration, which is not corrected by urinary creatinine levels, is 10.1ng/mL or below.
3. Accuracy of diagnosis
   As shown in Table 1 and Figure 1 the true negative ratio to the range of reference value (below 8.4 μg/gCr) of the CE-marked product was 100% (50/50), and the true positive ratio beyond the upper limit of the range of reference value was 68% (38/56).

Table 1 True positive rate and true negative rate

Assessment of normal or abnormal based on urine creatinine correction value	<12.5 (Negative)	≥12.5, <100	≥100
Abnormal (>8.4 μg/gCr)	18	35	3
Normal (≤8.4 μg/gCr)	50	0	0



Score 1: <12.5 Score 2: ≥12.5, <100 Score 3: ≥100

Figure 1 Relationship of the score of this product with urine L-FABP value (creatinine correction value) of ELISA (CE-marked product)

4. Result of correlation test
   When the correlation between this product and the CE-marked product "RENISCHEM® L-FABP TMB Kit" was evaluated, the following excellent correlation was obtained.

Urine		RENISCHEM® L-FABP POC Kit <12.5	RENISCHEM® L-FABP POC Kit ≥12.5, <100	RENISCHEM® L-FABP POC Kit ≥100	Total
RENISCHEM® L-FABP TMB Kit	<12.5	60	0	0	60
	≥12.5, <100	8	33	0	41
	≥100	0	3	31	34
Total		68	36	31	135

Concordance rate 91.9% (124/135)

When the correlation between this product and the CE-marked product "RENISCHEM®" was evaluated, the following excellent correlation was obtained.

Urine		RENISCHEM® L-FABP POC Kit <12.5	RENISCHEM® L-FABP POC Kit ≥12.5, <100	RENISCHEM® L-FABP POC Kit ≥100	Total
RENISCHEM®	<12.5	62	1	0	63
	≥12.5, <100	6	32	0	38
	≥100	0	3	31	34
Total		68	36	31	135

Concordance rate 92.6% (125/135)

Reference ranges

NOTE: The cut-off may vary according to clinical situations. Therefore, clinicians should use the L-FABP results in conjunction with other laboratory test results and clinical symptoms of the patient and interpreted the concrete values in the context with the clinical situation of the patient.

L-FABP < 12.5ng/mL
L-FABP ≥ 12.5ng/mL and <100ng/mL
L-FABP ≥ 100ng/mL

PERFORMANCE CHARACTERISTICS

1. Sensitivity
   When measuring the control samples prepared for sensitivity test (12.5ng/mL), control sample is classified into corresponding range of L-FABP concentration(close to or greater than 12.5ng/mL and less than 100ng/mL)on the reference card.
2. Accuracy
   When measuring the three control samples prepared for accuracy test (A.0-12.5ng/mL, B.12.5-100ng/mL, C.≥100ng/mL), each control samples is classified into each corresponding range of L-FABP concentrations (A.0-12.5ng/mL, B.12.5-100ng/mL, C.≥100ng/mL) on the reference card.
3. Repeatability
   When measuring the same sample for 3 times simultaneously, identical reactivity is confirmed.
4. Substance
   (1) Control sample for sensitivity test and accuracy test
   L-FABP standard 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 combined to adjust the density.

PACKAGE UNIT

10 Tests

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■GLOSSARY OF SYMBOLS

IVD	In Vitro Diagnostic Medical Device	DP	Dropper
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
TC	L-FABP Test Cassette		Manufacturer
		EC REP	Authorized representative in the European Community
PT	Pre-treatment Micro Tube	CONTENTS	Contents of kits

CMIC HOLDINGS Co.,Ltd.
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EC REP Emergo Europe
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