Getein Biotech, Inc.

CE

NGAL Fast Test Kit (Immunofluorescence Assay)
For *in vitro* Diagnostic Use

User Manual

INTENDED USE

NGAL Fast Test Kit (Immunofluorescence Assay) is intended for *in vitro* quantitative determination of neutrophils gelatinase associated lipocalin (NGAL) in urine. This test is used as an aid in the early diagnosis of acute kidney injury (AKI), risk classification and treatment monitoring.

SUMMARY

The inclining incidence of chronic kidney disease which has led to high mortality and immense medical burden over the past decades has become a distressing concern in epidemiology. Unfortunately, the number of biomarkers that allow the monitoring of chronic kidney disease (CKD) is limited. NGAL is an emerging biomarker which has been shown to be able to aid the diagnose of kidney injuries.

The evidence for the role of NGAL measurements in a variety of clinical situations leading to AKI (cardiac surgery, kidney transplantation, contrast nephropathy, haemolytic uraemic syndrome and in the intensive care setting) or to CKD (lupus nephritis, glomerulonephritides, obstruction, dysplasia, polycystic kidney disease, IgA nephropathy) is explored. The emerging utility of standardized clinical platforms for reliable measurement of NGAL in plasma (Triage NGAL Device; Biosite Incorporated) and urine (ARCHITECT analyzer; Abbott Diagnostics) is also discussed. It will be important in future studies to validate the sensitivity and specificity of NGAL concentration measurements in clinical samples from large cohorts and from multiple clinical situations. Such studies will be facilitated by the anticipated widespread availability of standardized commercial tools in the near future.

PRINCIPLE

The test uses an anti-human NGAL polyclonal antibody conjugated with fluorescence and an anti-human NGAL monoclonal antibody coated on the test line. After the sample has been applied to the test strip, the fluorescence-labelled anti-human NGAL polyclonal antibody binds with the NGAL in sample and forms an antigen-antibody complex. This complex moves to the test card detection zone by capillary action. Then marked antigen-antibody complex is captured on the test line by the anti-human NGAL monoclonal antibody. The fluorescence intensity of the test line increases in proportion to the amount of NGAL in sample.

Then insert test card into Getein1100 Immunofluorescence Quantitative Analyzer (hereafter referred to as Getein1100), the concentration of NGAL in sample will be measured and displayed on the screen. The value will be stored in Getein1100 and available for downloading. The result can be easily transmitted to the laboratory or hospital information system.

CONTENTS

A kit contains:

1. Getein NGAL test card in a sealed pouch with desiccant ····· 25
2. Disposable pipet ····· 25
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4. SD card ····· 1

A test card consists of:

A plastic shell and a reagent strip which is composed of a sample pad, nitrocellulose membrane (one end of the membrane is coated with a fluorescence-labelled anti-human NGAL polyclonal antibody, the test line is coated with an anti-human NGAL monoclonal antibody, and the control line is coated with rabbit anti-goat IgG antibody), absorbent paper and liner.

Note: Components from different batches must not be interchanged.

APPLICABLE DEVICE

Getein1100 Immunofluorescence Quantitative Analyzer

STORAGE AND STABILITY

Store the test card at 4~30°C with a valid period of 24 months.
Use the test card within 1 hour once the foil pouch is opened.

PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. For professional use only.
3. Do not use the kit beyond the expiration date.
4. Do not use the test card if the foil pouch is damaged.
5. Do not open pouches until ready to perform the test.
6. Do not reuse the test card.
7. Do not reuse the pipet.
8. Handle all specimens as potentially infectious. Proper handling and disposal methods should be followed in accordance with local regulations.
9. Carefully read and follow the manual to ensure proper test performance.

SAMPLE COLLECTION AND PREPARATION

1. This test can be used for urine sample.
2. Urine sample can be preserved at room temperature for 4 hours, please test it as soon as possible. If testing will be delayed, urine sample may be stored up to 3 days at 2~8°C prior to being tested.
3. Samples should be brought to room temperature prior to testing.
4. Do not use frozen urine samples.
5. Do not use heat-inactivated samples.
6. SAMPLE VOLUME: 100 µl.

TEST PROCEDURE

1. Collect specimen according to manual.
2. Test card, sample should be brought to room temperature prior to testing.
3. Confirm SD card lot No. in accordance with test kit lot No.. Perform “SD Card Calib” calibration when necessary (Details refer to 8.5.2 of Getein1100 User Manual).
4. On the main interface of Getein1100, press "ENT" button to enter testing interface.
5. Remove the test card from the sealed pouch immediately before use. Label the test card with patient or control identification.
6. Put the test card on a clean table, horizontally placed.
7. Using sample transfer pipette, deliver 100 µl of sample (or 3-4 drops of sample when using disposable pipet) into the sample port on the test card.
8. After 3 minutes, insert the test card into Getein1100 and press "ENT" button. The result will be shown on the screen and printed automatically.

Notes:

1. It is required to perform “SD Card Calib” calibration when using a new batch of kit.
2. It is suggested to calibrate once for one batch of kit.
3. Make sure the test card insertion is correct and complete.

TEST RESULTS

Getein1100 can scan the test card automatically and display the result on the screen. Please follow the procedure in user manual of Getein1100 for result printing.

For additional information, please refer to the user manual of Getein1100.

EXPECTED VALUE

The expected normal value for NGAL was determined by testing samples from 319 apparently healthy individuals. The 99th percentile of the concentration for NGAL is 135 ng/ml.

It is recommended that each laboratory establish its own expected values for the population it serves.

PERFORMANCE CHARACTERISTICS

Measuring Range: 50~1500 ng/ml
Lower Detection Limit: ≤50 ng/ml
Within-Run Precision: ≤10%
Between-Run Precision: ≤15%
Method Comparison:
The assay was compared to Abbott I2000 analyzer and its matching NGAL test kits with 200 urine samples (157 positive samples and 43 negative samples). The correlation coefficient (r) is 0.989.

LIMITATIONS

1. As with all diagnostic tests, a definitive clinical diagnosis should not be made based on the result of a single test. The test results should be

interpreted considering all other test results and clinical information such as clinical signs and symptoms.

2. Samples containing interferents may influence the results. The table below listed the maximum allowance of these potential interferents.

Interferent	Concentration (Max)
Creatinine	10 g/L
Glucose	10 g/L
Urea	100 g/L

REFERENCES

1. Wasilewska A, Taranta-Janusz K, Dębek W, et al. KIM-1 and NGAL: new markers of obstructive nephropathy. Pediatr Nephrol. 2011, 26(4): 579-586.
2. Clerico A, Galli C, Fortunato A, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as biomarker of acute kidney injury: a review of the laboratory characteristics and clinical evidences. Clin Chem Lab Med. 2012, 50(9): 1505-1517.
3. Shemin D, Dworkin LD. Neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker for early acute kidney injury. Crit Care Clin. 2011, 27(2): 379-389.
4. EN ISO 18113-1:2009 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 1: Terms, definitions and general requirements.
5. EN ISO 18113-2:2009 In vitro diagnostic medical devices - Information supplied by the manufacturer (labelling) - Part 2: *In vitro* diagnostic reagents for professional use (ISO 18113-2:2009).

DESCRIPTION OF SYMBOLS USED

The following graphical symbols used in or found on NGAL Fast Test Kit (Immunofluorescence Assay) are the most common ones appearing on medical devices and their packaging. They are explained in more detail in the European Standard EN 980: 2008 and International Standard ISO 15223-1:2007.

Key to symbols used			
	Manufacturer		Expiration Date
	Do not re-use		Date of manufacture
	Consult instructions for use	LOT	Batch code
	Temperature limitation	IVD	In vitro diagnostic medical device
	Sufficient for	EC REP	Authorized representative in the European Community
CE	CE marking		

Thank you for purchasing NGAL Fast Test Kit (Immunofluorescence Assay). Please read this manual carefully before operating to ensure proper use.

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Getein Biotech, Inc.
Add: No.9 Bofu Road, Luhe District, 211505 Nanjing, China
Tel: +86-25-68568508
Fax: +86-25-68568500
E-mail: tech@getein.com.cn overseas@getein.com.cn
Website: http://www.bio-gp.com.cn