AIS **Antibody and Immunoassay Services**

Li Ka Shing Faculty of Medicine, University of Hong Kong

The NGAL Turbidimetric Immunoassay Reagent Kit

Catalogue number: 51300

For the quantitative determination of NGAL
in human plasma and urine

This package insert must be read in its entirety before using this product
Use only the current version of product data sheet enclosed with the kit

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INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL), also known as Lipocalin-2 (LCN2), is a protein expressed in neutrophils and at low levels in the kidney. In the case of acute kidney injury (AKI), NGAL is secreted at high levels into the blood and urine within 2 hours of injury.

Renal health is most frequently assessed by measuring serum creatinine level, as serum creatinine is a marker of impaired renal function; whereas NGAL is a marker of kidney injury. NGAL levels are a more precise and sensitive marker for diagnosing AKI than serum creatinine levels. In fact, the increase in urinary excretion of NGAL has been proven to be due to tubular alterations that take place before any damage can be detected by other methods. Therefore, monitoring NGAL levels reduces delayed AKI diagnosis and treatment.

NGAL can also be used as an early diagnosis for chronic kidney disease, contrast-induced nephropathy and diabetic nephropathy.

PRINCIPLE OF THE ASSAY

This assay is a turbidimetric immunoassay for the quantitative measurement of NGAL in human serum and urine. A standard or sample is added into a cuvette and mixed with the reaction buffer R1. After a short incubation, the test reagent R2, which is a suspension of microparticles coated with NGAL antibodies, is added into the cuvette and mixed. The presence of NGAL in the standard or sample causes the immune-particles to aggregate. The extent to which the microparticles aggregate is quantified by the amount of light scattering measured as absorbance by a chemistry analyzer. The concentration of NGAL in unknown samples can be interpolated from a reference curve using the standards provided.

REAGENTS SUPPLIED

R1 – Reaction buffer, 45 ml, a ready-to-use buffer solution containing salt, polyether compound and preservative

R2 – Test reagent, 15 ml, a ready-to-use suspension of polymer microparticles coated with rabbit anti-NGAL polyclonal antibodies in storage buffer

NGAL Standard set – 5 tubes of NGAL standard: 100 ng/ml, 250 ng/ml, 1000 ng/ml, 2500 ng/ml, 5000 ng/ml, 1 ml per tube

NGAL Control –High and Low NGAL control，1ml per tube

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Clinical chemistry analyzer
2. Deionized water
3. Analyzer-specific reagent containers for R1 and R2

STORAGE

The kit should be stored at 2-8 ℃ upon receipt. Once opened, the reagents may be stored at 2-8 ℃ for up to 4 weeks.

SAMLE HANDLING

This kit can be used to determine NGAL in human urine and plasma samples. Blood specimens should be collected aseptically into appropriate tubes. Plasma should be prepared by standard techniques for laboratory testing. Urine should be centrifuged. The prepared specimens should be stored in closed vessels. If the assay cannot be performed within 24 hours or specimens are to be shipped, the specimens should be frozen at −20°C or below. For long-term storage of specimens, −70°C or below is recommended. To avoid freeze-thaw cycles, specimens should be aliquoted. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens. No dilution of the sample is required in this assay.

ASSAY PROCEDURE

Assay procedures may vary depending on the automated chemistry analyzer to be used. A general example of assay procedures is stated as follow:
1. Dispense 150 μl of R1 into a clean cuvette
2. Add 1.5μl of sample and incubate at 37˚C for 5 minutes
3. Further add 50 μl of R2
4. Read change of absorbance at 570 nm for 8 minutes after the addition of R2
5. Calculate the concentration of NGAL in unknown sample by interpolation from a reference curve using the standards provided

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

NGAL (ng/mL)	Absorbance (570 nm)
0	0
100	0.0139
250	0.0362
1000	0.1735
2500	0.4322
5000	0.6134
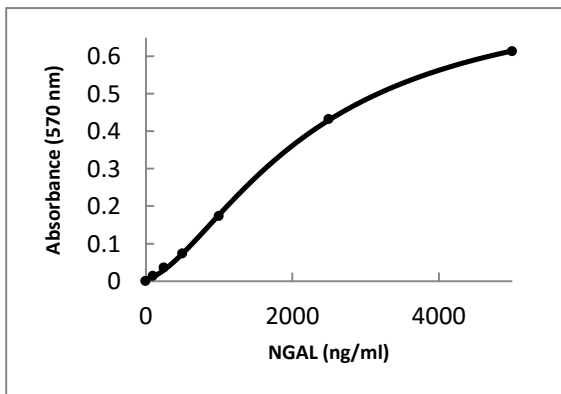
CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against NGAL concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. 4-parameter curve fitting can be used for calculation.
3. Determine NGAL concentration of samples from standard curve.

ASSAY CHARACTERISTICS

A. Sensitivity
The sensitivity is defined as the lower limit of detection and is estimated as the mean of the blank sample plus three times the SD obtained from the blank sample. The sensitivity of NGAL assay is 17ng/ml.

B. Precision
The precision of the NGAL assay is < 10% CV. Four samples consisting of two NGAL controls and two serum based panels were assayed 20 times separately.

Sample	Mean NGAL (ng/ml)	SD (ng/ml)	CV
Low control	93.7	7.3	7.77%
High control	198.5	8.9	4.46%
Panel 1	104.9	9.0	8.58%
Panel 2	156.1	3.8	2.44%

C. Linearity
The NGAL assay is linear between 50ng/ml and 5000 ng/ml.

D. Interference
No interference was detected with hemoglobin up to 5 g/L, conjugated bilirubin up to 300 mg/L, free bilirubin up to 300 mg/L, and up to 5g/L lipid emulsion.

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