# The Neutrophil Gelatinase-Associated Lipocalin (NGAL) Turbidimetric Immunoassay Kit

Catalogue number: 51050

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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**FOR RESEARCH USE ONLY**
**NOT FOR USE IN DIAGNOSTIC PROCEDURES**

Version:6.1

## TABLE OF CONTENT

## PACKING SPECIFICATION

| Cat. No. | Size | Approximately tests |
|---|---|---|
| 51050-05 | R1: 15ml, R2: 5ml | 100 |
| 51050-10 | R1: 30ml, R2: 10ml | 200 |
| 51050-20 | R1: 60ml, R2: 20ml | 400 |
| 51050-50 | R1: 150ml, R2: 50ml | 1000 |
| 51050-100 | R1: 300ml, R2: 100ml | 2000 |

## INTRODUCTION

Neutrophil gelatinase-associated lipocalin (NGAL), also known as Lipocalin-2 (LCN2), is a protein expressed in neutrophils and in the kidney at low levels in healthy subjects. During acute kidney injury (AKI), NGAL is secreted at high levels into the bloodstream and urine within 2 hours of injury. Therefore, NGAL is a more accurate and sensitive biomarker for diagnosing AKI than serum creatinine. In fact, the increase in urinary excretion of NGAL has been proven to be due to tubular alterations that take place well 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 biomarker for diagnosis of chronic kidney disease, contrast-induced nephropathy and diabetic nephropathy. The reference value of the NGAL in plasma is < 180 ng/mL.

IMD developed NGAL PETIA kits with its highly specific anti-human NGAL antibodies and recombinant human NGAL.

## PRINCIPLE OF THE ASSAY

This assay is a turbidimetric immunoassay for the quantitative measurement of NGAL in human plasma 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, a ready-to-use buffer solution containing salt, polyether compound and preservative

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

## OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Clinical chemistry analyzer
2. NGAL calibrator, provided separately (Cat No: 51050-S1)
3. NGAL quality controls , optional, provided separately (51050-C1)
4. Deionized water
5. Analyzer-specific reagent containers for R1 and R2

## STORAGE

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

## SPECIMEN COLLECTION AND 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

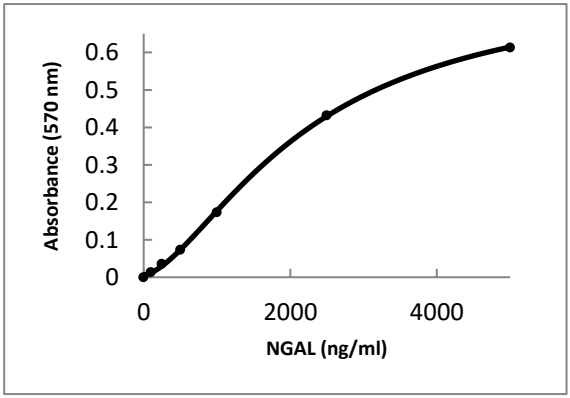
Assay procedures may vary depending on the automated chemistry analyzer to be used. A general example of assay procedures is stated as follow:

1. Dispense 150µl of R1 into a clean cuvette
2. Add 1.5µl of sample and incubate at 37˚C for 5 minutes
3. Further add 50µl 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.

## PERFORMANCE 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 plasma samples were assayed 20 times separately.

| Sample | Mean NGAL (ng/ml) | SD (ng/ml) | CV |
|---|---|---|---|
| Low control | 172 | 5 | 2.78% |
| High control | 516 | 9 | 1.75% |
| Plasma 1 | 132 | 10 | 7.17% |
| Plasma 2 | 141 | 7 | 5.25% |

### 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.

## EXPECTED VALUES

Reference range: Plasma sample: 0 – 180 ng/ml

## WARNINGS AND PRECAUTIONS

- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Wear protective clothing and disposable gloves while handling the kit reagents.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes; use safety glasses; in case of contact, flush with water immediately and contact a doctor.
- Avoid contact with skin; use gloves; in case of contact with skin, flush immediately and thoroughly with water.
- Dispose of all specimens and components of the kit as potentially infectious agents.
- Do not use the kit or any kit component past the indicated expiry date.
- Do not use any other reagents from different lots in this test, unless the reagent is designated to be used with other lots of the same kit.
- Do not use any reagent in other test kits, unless the reagent is designated to be used with other kits.
- Avoid microbial contamination of reagents.
- For manual pipetting of samples and controls, use individual pipette tips to eliminate carryover.

## REFERENCES

1. Binbin Wu, Jianghua Chen, et al. Biomarkers of Acute Kidney Injury after Cardiac Surgery: A Narrative Review. *Biomed Res Int.* 2019 Jun 27;2019:7298635.