# Human NGAL Sandwich ELISA Kit Datasheet

**Catalogue Number:** KE00144

**Size:** 5*96T

**Sensitivity:** 3.3 pg/mL

**Range:** 78.1-5000 pg/mL

**Usage:** For the quantitative detection of human NGAL concentrations in serum, plasma, cell cuture supernatant, urine and saliva.
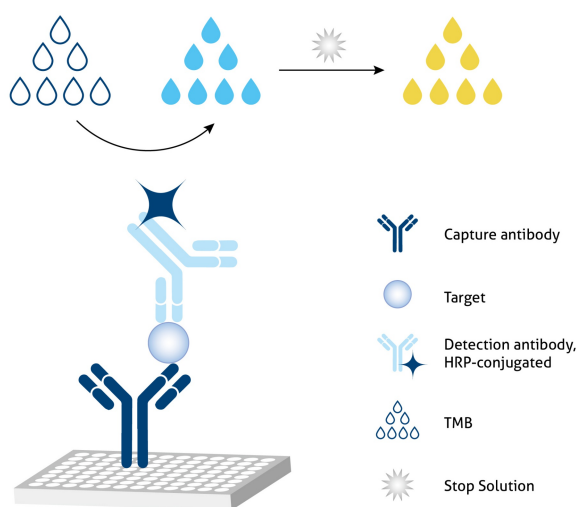
**This product is for research use only and not for use in human or animal therapeutic or diagnostic.**

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# 1. Background

Neutrophil gelatinase-associated lipocalin (NGAL) also known as Lipocalin-2 (LCN2), is a secreted glycoprotein that belongs to the lipocalin protein family. NGAL exists as a monomer, a homodimer and a disulfide-linked heterodimer in cells, covalently conjugated with gelatinase (matrix metalloproteinase-9, MMP-9) in order to decrease its degradation. Several roles of NGAL was identified, including iron trafficking, chemotactic, and promote cell differentiation and proliferation. NGAL was originally found in human neutrophils, and expressed in tissues containing renal, uterus, liver, spleen, as well as cardiomyocytes, neurons and immune cells. Studies showed that NGAL is a significant biomarker of cardiovascular diseases, ischemic stroke, acute renal injury, inflammation and metabolic diseases, and it can be detected in human serum, saliva and urine.

# 2. Principle



| | |
|---|---|
| Capture antibody | |
| Target | |
| Detection antibody, HRP-conjugated | |
| TMB | |
| Stop Solution | |

**Sandwich ELISA structure (Detection antibody labeled with HRP)**

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

# 3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

# 4. Kit Components and Storage

| | | |
|---|---|---|
| **Microplate** - antibody coated 96-well microplate (8 well × 12 strips) | 5 plates | **Unopened Kit:** |
| **Protein standard** - 10000 pg/bottle; lyophilized | 10 bottles | |
| **Detection antibody, HRP-conjugated (100×)** - 600 µL/vial* | 1 vial | Store at 2-8°C for 6 months or -20°C for 12 months. |
| **Sample Diluent PT 1** - 150 mL/bottle | 1 bottle | |
| **Detection Diluent** - 150 mL/bottle | 1 bottle | **Opened Kit:** |
| **Wash Buffer Concentrate (20×)** - 150 mL/bottle | 1 bottle | All reagents stored at 2-8°C for 7 days. |
| **Tetramethylbenzidine Substrate (TMB)** - 60 mL/bottle | 1 bottle | |
| **Stop Solution** - 60 mL/bottle | 1 bottle | **Please use a new standard for each assay.** |
| **Plate Cover Seals** | 15 pieces | |

* Centrifugation immediately before use

# 5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

# 6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

6.4 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

6.5 Saliva: Collect saliva samples and centrifuge for 5 minutes at 10,000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

# 7. Regent Preparation

**7.1 Wash Buffer (1X):** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.
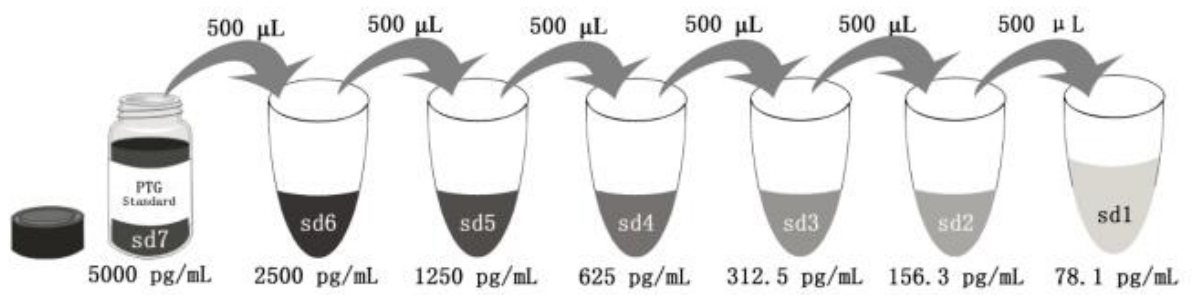
**7.2 Detection Antibody, HRP-conjugated (1X):** Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Detection Antibody, HRP-conjugated + 990 μL Detection Diluent (Centrifuge the 100 X Detection Antibody solution, HRP-conjugated for a few seconds prior to use)

**7.3 Sample Dilution:** Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:40 or 1:80 is recommended for human serum, human plasma and saliva; 1:8 or 1:16 is recommended for cell culture supernatant; 1:4 or 1:8 is recommended for urine.

**7.4 Standard Serial Dilution:**

Add 2 mL Sample Diluent PT 1 in protein standard.



| | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
|---|---|---|---|---|---|---|
| sd7 | sd6 | sd5 | sd4 | sd3 | sd2 | sd1 |
| 5000 pg/mL | 2500 pg/mL | 1250 pg/mL | 625 pg/mL | 312.5 pg/mL | 156.3 pg/mL | 78.1 pg/mL |

| Add # μL of Standard diluted in the previous step | — | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
|---|---|---|---|---|---|---|---|
| # μL of Sample Diluent PT 1 | 2000 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL | 500 μL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

# 8. Assay Procedure Summary

**Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.**

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 μL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 μL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Signal development: Add 100 μL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.8 Quenching color development: Add 100 μL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.9 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.10 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

| Step | Reagent | Volume | Incubation | Wash | Notes |
|------|---------|--------|-----------|------|-------|
| 1 | Standard and Samples | 100 μL | 120 min | 4 times | Cover Wells incubate at 37°C |
| 2 | Diluent Detection Antibody, HRP-conjugated Solution | 100 μL | 40 min | 4 times | Cover Wells incubate at 37°C |
| 3 | TMB Substrate | 100 μL | 15-20 min | Do not wash | Incubate in the dark at 37°C |
| 4 | Stop Solution | 100 μL | 0 min | Do not wash | - |
| 5 | Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes. | | | | |

# 9. Validation Data

## 9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.
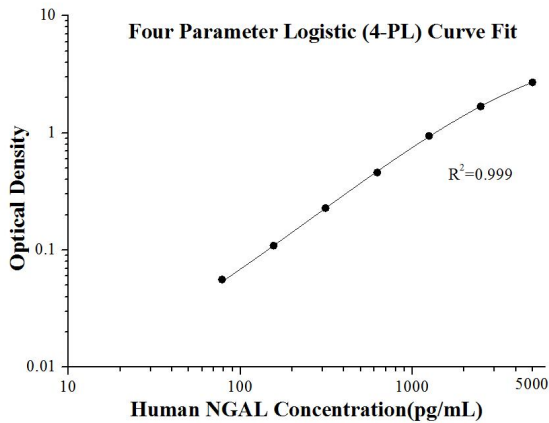


| (pg/mL) | O.D | Average | Corrected |
|---|---|---|---|
| 0 | 0.027 0.026 | 0.027 | - |
| 78.1 | 0.083 0.081 | 0.082 | 0.056 |
| 156.2 | 0.133 0.138 | 0.136 | 0.109 |
| 312.5 | 0.256 0.252 | 0.254 | 0.228 |
| 625 | 0.483 0.488 | 0.486 | 0.459 |
| 1250 | 0.968 0.967 | 0.968 | 0.941 |
| 2500 | 1.551 1.863 | 1.707 | 1.681 |
| 5000 | 2.704 2.747 | 2.726 | 2.699 |

## 9.2 Precision

**Intra-assay Precision** (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

| Intra-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 20 | 2,493.7 | 166.0 | 6.7 |
| 2 | 20 | 655.1 | 16.6 | 2.5 |
| 3 | 20 | 154.8 | 11.0 | 7.1 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 24 | 2,550.7 | 78.4 | 3.1 |
| 2 | 24 | 625.4 | 17.5 | 2.8 |
| 3 | 24 | 150.1 | 7.4 | 4.9 |

## 9.3 Recovery

The recovery of human NGAL spiked to three different levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | | Average % of Expected | Range (%) |
|---|---|---|---|
| Human serum | 1:160 | 97 | 89-106 |
| | 1:320 | 88 | 79-96 |
| Cell culture supernatant | 1:32 | 89 | 74-99 |
| | 1:64 | 87 | 73-103 |
| Urine | 1:16 | 91 | 84-102 |
| | 1:32 | 92 | 86-104 |
| Saliva | 1:160 | 97 | 88-112 |
| | 1:320 | 97 | 75-113 |

## 9.4 Sample values

Serum, plasma samples from healthy volunteers were evaluated for NGAL in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean(ng/mL) | Rang (ng/mL) |
|---|---|---|
| Human serum (n=12) | 158.0 | 113.2-228.7 |
| Urine (n=8) | 12.3 | 0.4-28.5 |
| Saliva(n=8) | 245.0 | 87.1-304.4 |

Human peripheral blood mononuclear cells ($1 \times 10^6$ cells/mL) were cultured in RPMI supplemented with 8% fetal bovine serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. Cells were stimulated with 10 ug/mL PHA. Aliquots of the cell culture supernatant was removed on days 1 and 3 and assayed for levels of human NGAL.

| Condition | Day 1 (ng/mL) | Day 3 (ng/mL) |
|---|---|---|
| Unstimulated | 7.9 | 6.7 |
| Stimulated | 10.5 | 9.0 |

## 9.5 Sensitivity

The minimum detectable dose of human NGAL is 3.3 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

## 9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The human serum and saliva samples were initially diluted 1:20. The cell culture supernatant samples were initially diluted 1:4. The urine samples were initially diluted 1:2. )

| | | Human serum | Cell culture supernatant | urine | saliva |
|---|---|---|---|---|---|
| 1:2 | Average% of Expected | 100 | 100 | 100 | 100 |
| | Range (%) | - | - | - | - |
| 1:4 | Average% of Expected | 99 | 107 | 99 | 107 |
| | Range (%) | 93-105 | 103-111 | 96-101 | 105-108 |
| 1:8 | Average% of Expected | 108 | 112 | 99 | 110 |
| | Range (%) | 99-117 | 106-119 | 98-99 | 103-118 |
| 1:16 | Average% of Expected | 124 | 107 | 92 | 110 |
| | Range (%) | 118-133 | 82-130 | 90-94 | 102-118 |

## 10. References

1. Bauvois B. et al. (2018). Cancers (Basel). 18;10(9).

2. Buonafine M. et al. (2018). Clin Sci (Lond). 132(9):909-923.

3. Abella V. et al. (2015). Biomarkers. 20(8):565-71.

Devarajan P. et al. (2008). Scand J Clin Lab Invest Suppl. 241:89-94.