# HUMAN NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) / LIPOCALIN-2 ELISA KIT

**FOR THE QUANTITATIVE DETERMINATION OF HUMAN NGAL CONCENTRATIONS IN URINE, SERUM AND PLASMA**

**ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.**

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

**PURCHASE INFORMATION:**

| **ELISA NAME** | **HUMAN NGAL/LIPOCALIN-2 ELISA** |
|---|---|
| Catalog No. | SK00233-01 |
| Lot No. | |
| Formulation | 96 T |
| Standard range | 19.5 - 1250 pg/mL |
| Sensitivity | 10 pg/mL |
| Sample Volume | 100 µL |
| Dilution | 50 for serum or plasma. 10 for urine. **(*Optimal dilutions should be determined by each laboratory for each application*)** |
| Sample Type | Serum, EDTA Plasma, Urine |
| Specificity | Human NGAL |
| Calibration | Human NGAL recombinant |
| Intra-assay Precision | 4 - 6% |
| Inter-assay Precision | 8 - 10% |
| Storage | 2 – 8° C |
| This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol. | |

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## DESCRIPTION

This Human NGAL/Lipocalin-2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human NGAL/Lipocalin-2 from urine, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human NGAL/Lipocalin-2 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural NGAL/Lipocalin-2 samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human NGAL/Lipocalin-2. The capture antibody can bind to the human NGAL/Lipocalin-2 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human NGAL/Lipocalin-2 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human NGAL/Lipocalin-2 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

| DESCRIPTION | CODE | QUANTITY |
|---|---|---|
| **NGAL Microplate** - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human NGAL. | **233-01-01** | **1 plate** |
| **NGAL Standard** – 10000 pg/vial of recombinant human NGAL in a buffered protein base with preservative; lyophilized. | **233-01-02** | **1 vial** |
| **Detection Antibody Concentrate** – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against human NGAL with preservative; lyophilized. | **233-01-03** | **1 vial** |
| **Positive Control** - one vial of recombinant human NGAL; lyophilized (optional). | **233-01-04** | **1 vial** |
| **Streptavidin-HRP Conjugate** - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP. | **SAHRP** | **1 vial** |
| **Dilution Buffer** - 60mL of buffered protein based solution with preservative. | **DB18** | **1 bottle** |
| **Antibody Diluent Solution** – 12 mL of buffered protein based solution with preservative. | **DB08** | **1 bottle** |
| **HRP Diluent Solution** - 12 mL of buffered protein based solution with preservative. | **DB06** | **1 bottle** |
| **Wash Buffer** - 50 mL of 10-fold concentrated buffered surfactant, with preservative. | **WB01** | **1 bottle** |
| **TMB Substrate Solution** - 11 mL of TMB substrate solution. | **TMB01** | **1 bottle** |
| **Stop Solution** – 11 mL of 0.5M HCl. | **S-STOP** | **1 bottle** |
| **Plate Sealer** | **EAPS** | **1 piece** |
| **Plastic Pouch** | **P01** | **1 piece** |

## STORAGE

**Unopened Kit:** Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
**Opened / Reconstituted Reagents:** Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 6 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8° C for up to 6 months.
**Microplate Wells:** Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

## ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

## PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

## SAMPLE COLLECTION AND STORAGE

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
**EDTA Plasma** - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
**Notice:** Heparin can't be used as anticoagulant for NGAL assay.
**Urine** - Collect the first urine of the day (mid-part). Centrifuge to remove particulates, assay immediately or aliquot and store at -20° C ~-70° C. Avoid repeated freeze-thaw cycles.
**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

## SAMPLE PREPARATION

Serum and plasma samples may require a 50-fold dilution. A suggested 50-fold dilution is 5 µL sample + 245 µL Dilution Buffer. Urine samples may require a 10-fold dilution. A suggested 10-fold dilution is 25 µL sample + 225 µL Dilution Buffer.
**Optimal dilutions should be determined by each laboratory for each application.**
**Use polypropylene test tubes.**

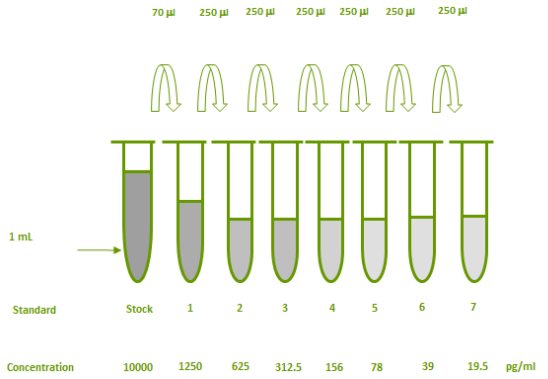
## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

**NGAL Standard -** Reconstitute the human NGAL standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 490 µL of Dilution Buffer into tubes #1.Transfer 70 µL of stock solution to make a solution of 1250 pg/mL. Pipette 250 µL of Dilution Buffer into tubes #2 to #7. Use the **1250 pg/mL** stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1250 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

| TUBE | STANDARD | DILUTION BUFFER | CONCENTRATION |
|---|---|---|---|
| stock | Powder | 1000 µl | 10000 pg/ml |
| # 1 | 70 µl of stock | 490 µl | 1250 pg/ml |
| # 2 | 250 µl of 1 | 250 µl | 625 pg/ml |
| # 3 | 250 µl of 2 | 250 µl | 312.5 pg/ml |
| # 4 | 250 µl of 3 | 250 µl | 156.25 pg/ml |
| # 5 | 250 µl of 4 | 250 µl | 78.125 pg/ml |
| # 6 | 250 µl of 5 | 250 µl | 39.063 pg/ml |
| # 7 | 250 µl of 6 | 250 µl | 19.531 pg/ml |




**Positive Control** - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used within a few days if stored at -20° C or -70° C.

**Detection Antibody -** Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB08)** to produce a 10-fold concentrated stock solution. Pipette 9.45mL of Antibody Diluent Solution (DB08) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

**Streptavidin-HRP Conjugate** - Transfer 120 µL of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of **HRP Diluent Solution (DB06)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of **Dilution Buffer** to Blank wells.
4. Add 100 µL per well of **Standard solutions** in reverse order of serial dilution, **sample**, or **positive control**. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **TMB Substrate Solution** to each well. Incubate for 5-10 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

## CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

| STANDARD (PG/ML) | AVERAGE OD450 (CORRECTED) |
|---|---|
| Blank | 0 (0.089) |
| 19.531 | 0.021 |
| 39.063 | 0.074 |
| 78.125 | 0.172 |
| 156.25 | 0.335 |
| 312.50 | 0.586 |
| 625 | 0.941 |
| 1250 | 1.379 |

- Lot No.:
- Positive Control:

## SPECIFICITY

Human NGAL ELISA recognizes recombinant and natural Human NGAL.

| PROTEINS | CROSS-REACTIVITY (%) |
|---|---|
| Human NGAL rec. | 100% |
| Human MMP-9/NGAL | 0 |
| Mouse NGAL | 0 |
| Rat NGAL | 0 |
| Human KIM-1 | 0 |
| Human ECP | 0 |

## LINEARITY

To assess the linearity of the assay pooled research human **serum** samples were diluted with Dilution Buffer DB06 and assayed.

| DILUTION FACTOR | ASSAYED (PG/ML) | FINAL (NG/ML) | RECOVERY (%) |
|---|---|---|---|
| 50X | 608.168 | 30.4084 | 100 |
| 100X | 281.737 | 28.1737 | 92.7 |
| 200X | 114.558 | 22.9116 | 75.3 |

To assess the linearity of the assay pooled research human **EDTA plasma** samples were diluted with Dilution Buffer DB06 and assayed.

| DILUTION FACTOR | ASSAYED (PG/ML) | FINAL (NG/ML) | RECOVERY (%) |
|---|---|---|---|
| 50X | 898.165 | 44.9084 | 100 |
| 100X | 458.524 | 45.8524 | 102 |
| 200X | 217.098 | 43.4196 | 96.7 |

To assess the linearity and recovery of the assay, the research samples from healthy volunteers containing natural NGAL were serially diluted with Dilution Buffer DB06 and were tested by NGAL/Lipocalin-2 (Human) ELISA Kit SK00233-01.

| SAMPLE TYPE | DILUTION FACTOR | ASSAYED (PG/ML) | FINAL (NG/ML) | RECOVERY (%) |
|---|---|---|---|---|
| Human Urine | 20 x | 1020.494 | 20.409 | 100 |
| Human Urine | 40 x | 488.147 | 19.526 | 96 |
| Human Urine | 80 x | 247.408 | 19.793 | 97 |
| Human Urine | 160 x | 122.247 | 19.560 | 96 |

## REFERENCES:

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2: Shaker O, El-Shehaby A, El-Khatib M. Early Diagnostic Markers for Contrast Nephropathy in Patients Undergoing Coronary Angiography. Angiology. 2010 Jun 7. [Epub ahead of print].

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4: Allison SJ. Lupus nephritis: Urinary NGAL predicts renal flares in lupus nephritis. Nat Rev Nephrol. 2010 May;6(5):250.

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## SUMMARY OF ASSAY PROCEDURE

| PREPARE REAGENTS, SAMPLES AND STANDARDS |
|---|
|  |
| Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT. |
|  |
| Aspirate and wash 4 times. |
|  |
| Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT. |
|  |
| Aspirate and wash 4 times. |
|  |
| Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.** |
|  |
| Aspirate and wash 4 times. |
|  |
| Add 100 µl Substrate Solution to each well. Incubate 5-10 minutes on the plate shaker at RT. **Protect from light.** |
|  |
| Add 100 µl Stop Solution to each well. Read 450nm within 15 min. |