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

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

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	78-5000 pg/mL
Sensitivity	39 pg/mL
Sample Volume	100 µL
Dilution	50 for serum or plasma. 20-40 for urine (*Optimal dilutions should be determined by each laboratory for each application*)
Sample Type	Serum, EDTA Plasma, Urine
Specificity	Human NGAL
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2 - 8 °C

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INTRODUCTION

Human NGAL/Lipocalin-2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure NGAL in urine, serum and EDTA plasma. It contains recombinant NGAL and antibodies raised against this protein. It has been shown to accurately quantify recombinant NGAL. Results obtained with naturally occurring NGAL samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural NGAL.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for NGAL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any NGAL present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for NGAL is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of NGAL bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS 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 – 5000 pg/vial of recombinant Human NGAL in a buffered protein base with preservatives; lyophilized.	**233-01-02**	**1 vial**
Detection Antibody Concentrate – 600 µL/vial, 10-fold concentrated of biotinylated antibody against Human NGAL with preservatives; lyophilized.	**233-01-03**	**2 vials**
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 preservatives	**DB06**	**1 bottle**
Wash Buffer - 50 ml of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
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 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months. Reconstituted

Positive Control should be prepared and used within a few days.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 ml and 500 ml graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 -20ºC ~-70ºC. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

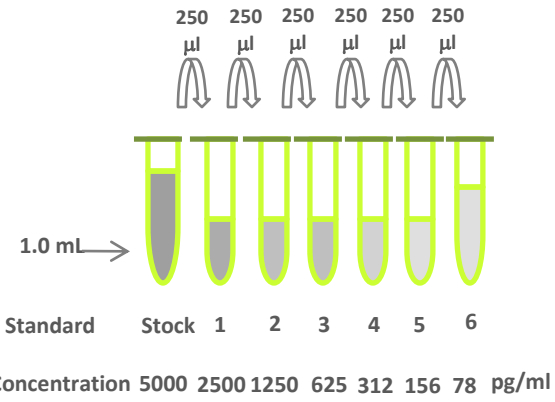
SAMPLE PREPARATION

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 20 or 40-fold dilution. A suggested 20-fold dilution is 15 µL sample + 285 µL Dilution Buffer DB06. A suggested 40-fold dilution is 7.5 µL sample + 292.5 µL Dilution Buffer DB06.
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 Wash Buffer.
NGAL Standard - Refer to vial label for reconstitution volume. Reconstitute the Human NGAL Standard with 1.0 ml of Dilution Buffer. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 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	5000 pg/ml
# 1	250 µl of stock	250 µl	2500 pg/ml
# 2	250 µl of 1	250 µl	1250 pg/ml
# 3	250 µl of 2	250 µl	625 pg/ml
# 4	250 µl of 3	250 µl	312.5 pg/ml
# 5	250 µl of 4	250 µl	156.25 pg/ml
# 6	250 µl of 5	250 µl	78.125 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 600 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 5.4 ml of Dilution Buffer into a 15 ml centrifuge tube and transfer 600 µl of 10-fold concentrated stock solution to prepare working solution. **Note:** This is enough for half a plate, included in this kit is two vials of Detection Antibody Concentrate.

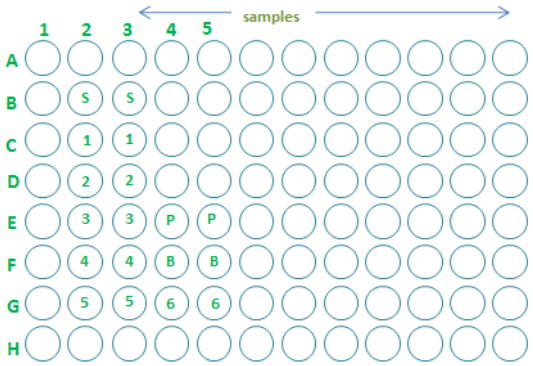
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated Streptavidin-HRP conjugate stock solution to 11.88 mL of Dilution Buffer to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

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 duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of **Dilution Buffer** to Blank well (F4, F5).
4. Add 100 µL of **Standard** (B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 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 sealer. Incubate for 2 hours on micro-plate 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 micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 7-11 minutes 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 micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the standard concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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 standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.117)
78.125	0.051
156.25	0.087
312.50	0.146
625	0.260
1250	0.449
2500	0.681
5000	1.102

*Lot No.:

** Positive Control: 500 – 1000 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant Human NGAL.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Human NGAL was 15 pg/mL.

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

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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 7-11 minutes on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min