RAT NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) / LIPOCALIN 2 (LCN2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION RAT NGAL CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURES.

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

PURCHASE INFORMATION:

ELISA NAME	RAT NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) ELISA KIT
Catalog No.	SK00233-09
Lot No.:	
Formulation	96 T
Standard range	0.78 ~ 50 ng/mL
Sensitivity	50 pg/mL
Sample Volume	100 µl
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum, EDTA plasma, cell culture
Specificity	Rat NGAL
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

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INTRODUCTION

Rat Neutrophil Gelactinase-Associated Lipocalin (NGAL)/Lipocalin-2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure NGAL in cell culture supernates, serum, and EDTA plasma. It contains recombinant rat NGAL and antibodies raised against this protein. It has been shown to accurately quantitie recombinant rat 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. A monoclonal antibody specific for Rat NGAL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any soluble NGAL present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for rat NGAL is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add 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

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ 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 the appropriate 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.

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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Rat NGAL Microplate – 96 well microplate precoated with anti-rat NGAL momoclonal antibody, one plate	233-09-01	1 plate
Rat NGAL Standard – 50 ng/vial of recombinant rat NGAL in a buffered protein base with preservatives; lyophilized.	233-09-02	1 vial
NGAL Antibody Concentrate– 120µl / vial, 100-fold concentrated of Biotinylated Antibody against rat NGAL with preservatives; lyophilized.	233-09-03	1 vial
Positive Control – one vial of recombinant Human VLP1, lyophilized (optional)	233-09-04	1 vial
Streptavidin-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	SAHRP	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB18	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered	WB01	1 vial

surfactant, with preservative.		
TMB Substrate Solution- 11ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution（0.5M HCl）, 11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer – Plate sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrated should be stored at -20 or -70 °C. Do not use past kit expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Positive Control and Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one months. Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 4 months at 2 - 8° C.

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.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
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.
Plasma - Collect plasma using EDTA, heparin, or citrate 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.
All samples collection should be used Aprotinin at 0.5 TIU per ml solution to protect NGAL/Lipocalin-2.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

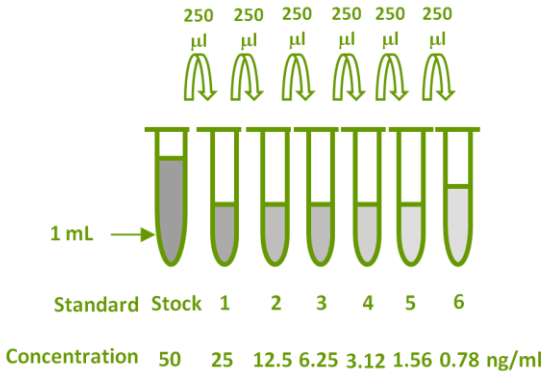
REAGENT PREPARATION

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.

Rat NGAL Standard - Refer to vial label for reconstitution volume. Reconstitute the **rat NGAL** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 50 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

STANDARD TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	50 ng/ml
# 1	250µl of stock	250µl	25 ng/ml
# 2	250µl of 1	250µl	12.5 ng/ml
# 3	250µl of 2	250µl	6.25 ng/ml
# 4	250µl of 3	250µl	3.125 ng/ml
# 5	250µl of 4	250µl	1.56 ng/ml
# 6	250µl of 5	250µl	0.78 ng/ml

VLP1Antibody- Reconstitute the **Antibody concentrated** with 120 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Transfer it to 11.88 mL of Dilution Buffer to prepare 1 x Antibody solution.



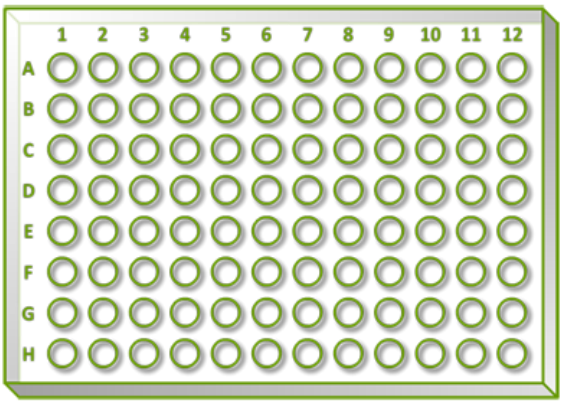
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 12 ml of Dilution Buffer to prepare working solution. Note: 1 x working solution of Streptavidin HRP Conjugate should be used within a few days.

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

ASSAY PROCEDURE

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

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 foil pouch containing the desiccant pack, reseal.
3. Leave well A2 and A3 as Blank. Add 100 µl per well of Dilution Buffer.
4. Add 100 µl per well of standard solution from #6 to #S (reverse order of serial dilution) to the appropriate wells (B2, B3 to G2,G3 and G4,G5). Add 100 µl per well of Positive control into well F4 and F5. Add 100 µl per well of samples into appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). Note: Standard, Blank and PC should be assayed in duplicate.
5. Aspirate wells and wash 4 times with 300 µl of 1 x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1 x Antibody solution. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate** working solution. Cover or seal the plate and incubate at room temperature for 45 minutes on microplate shaker.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL of Substrate Solution to each well. Incubate for 17-20 minutes at room temperature. **Protect from light**.
13. 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, QC, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a log-log curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant rat NGAL.

SENSITIVITY

The minimum detectable dose (MDD) of Rat NGAL Was 50 pg/mL.

TYPICAL DATA

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

STANDARD (NG/ML)	OD450 READING
Blank	0 (0.077)
0.78	0.060
1.56	0.157
3.125	0.349
6.25	0.662
12.5	0.750
25	1.068
50	1.467

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Rat NGAL	100
Mouse NGAL	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl 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 Antibody Solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Add 100 µl Streptavidin HRP conjugate to all wells. Incubate 45 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 17-20 min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min