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

FOR THE QUANTITATIVE DETERMINATION OF NGAL CONCENTRATIONS IN RAT AND MOUSE SERUM AND PLASMA

PRODUCT INFORMATION:

ELISA NAME	RAT/MOUSE NGAL ELISA
Catalog No.	SK00233-06
Lot No.	
Formulation	96 T
Standard Range	0.0128 - 1000 ng/mL
Dynamic Range	0.064 – 40 ng/mL
Sensitivity	0.0128 ng/mL
Sample Volume	50 µl of diluted samples
Dilution Factor	**Mouse:** no dilution is necessary **Rat:** 10-fold dilution **Optimal dilutions should be determined by each laboratory for each application with pretest**
Sample Type	Serum, EDTA plasma
Specificity	Rat and Mouse NGAL
Calibration	NGAL Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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.

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DESCRIPTION

This Rat/Mouse NGAL ELISA kit contains the necessary components required for the quantitative measurement of recombinant and/or natural rat and mouse NGAL from serum and plasma in a competitive EIA format.

This immunoassay contains recombinant and biotinylated recombinant NGAL and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural rat and mouse NGAL.

ASSAY OVERVIEW

This assay employs the quantitative competitive EIA format. NGAL present in samples compete with a fixed amount of biotinylated NGAL for sites on an antibody specific against NGAL. After a wash to remove any unbound standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of NGAL bound in the initial step. 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.
_ 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.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate - 96 well microplate pre-coated with polyclonal anti rabbit IgG Fc.	**RM01**	**1 plate**
NGAL Standard – 1000 ng/vial of recombinant NGAL in a buffered protein base with preservative; lyophilized.	**233-06-01**	**1 vial**
Biotin Concentrate – 600 µL/vial, 10-fold concentrate of NGAL biotinylated with preservative; lyophilized.	**233-06-02**	**1 vial**
Antibody Concentrate – 600 µl/vial, 10-fold concentrate of polyclonal purified IgG against NGAL with preservative; lyophilized.	**233-06-03**	**1 vial**
Positive Control – one vial of recombinant NGAL; lyophilized (optional).	**233-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin-HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB18**	**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 6 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (1000 ng/mL), Biotin concentrated solution and Antibody concentrated solution SHOULD BE

STORED at -20° C or -70° C for up to one month. Reconstituted Biotin Solution (600 µL) CAN NOT BE STORED at 2 – 8° C. Streptavidin-HRP Conjugate 100-fold concentrated solution (**protect from light**) and other components may be stored at 2 – 8° C for up to 6 months.

Microplate Wells: Return unused wells 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 measuring absorbance at 450 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.

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.

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.

OPTIONAL: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Mouse samples may not need to be diluted. Rat samples may need to be diluted 10-fold. A suggested 10-fold dilution is 15 µL sample + 135 µL Dilution Buffer**. Optimal dilutions should be determined by each laboratory for each application with pretest.**

Use polypropylene test tubes.

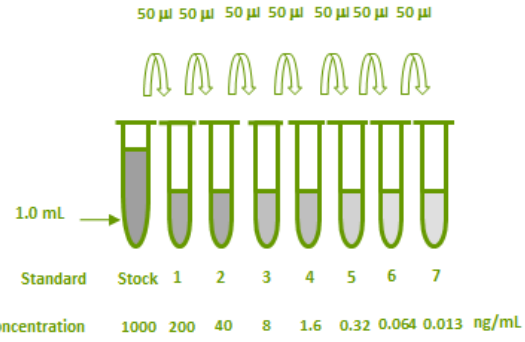
REAGENT PREPARATION

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 NGAL Standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 ng/mL** standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1000 ng/ml
# 1	50µl of stock	200µl	200 ng/ml
# 2	50µl of 1	200µl	40 ng/ml
# 3	50µl of 2	200µl	8 ng/ml
# 4	50µl of 3	200µl	1.6 ng/ml
# 5	50µl of 4	200µl	0.32 ng/ml
# 6	50µl of 5	200µl	0.064 ng/ml
# 7	50µl of 6	200µl	0.0128 ng/ml



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

Antibody Solution - Reconstitute the Antibody Concentrate with 600 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 5.4 mL of Dilution Buffer to prepare 1x Antibody solution.

Biotin Solution – Reconstitute the Biotin Concentrate with 600 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 5.4 mL of Dilution Buffer to prepare 1x Biotin solution.

Streptavidin-HRP Conjugate – Pipette 11.88 mL of **HRP Diluent Solution (DB06)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days (protect from light).*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

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. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTINYLATED SOLUTION INTO BLANK WELLS.***
4. Set two wells as total binding (TB). Add 50 µl per well of **Dilution Buffer**.
5. Add 50 µl per well of **Standard dilutions** from #7 to #S (reverse order of serial dilution) to the appropriate wells. Add 50 µl per well of **Positive Control** into appropriate wells. Add 50 µl per well of **samples** into appropriate wells.
6. Add 50 µl per well of **1x Antibody Solution** into total binding, standard dilutions, Positive Control and sample wells. Cover with plate sealer and incubate on microplate shaker (250 – 300 rpm) at room temperature for 2 hours. **Note: Do Not Aspirate and Wash Plate. Proceed immediately to the next step.**
7. Add 50 µl per well of **1x Biotin Solution** into total binding, standard dilutions, Positive Control and sample wells. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker. ***Note: DO NOT ADD Biotin Solution to Blank wells.***
8. Aspirate wells and wash 4 times with 300 µl of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate on microplate shaker for 60 minutes at room temperature. **Protect from light.**
10. Aspirate and wash as step 8.
11. Add 100 µl of **Substrate Solution** to each well. Incubate for 1-3 minutes on microplate shaker at room temperature. **Protect from light**.
12. 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.
13. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard dilutions, Positive Control and samples, and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) 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.

SPECIFICITY

Proteins	Cross-reactivity
Mouse NGAL	100%
Rat NGAL	100%

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.063)
TB	1.742
0.0128	1.645
0.064	1.557
0.32	1.618
1.6	1.299
8	0.701
40	0.310
200	0.095
1000	0.031

- Lot No.:
- Positive Control:

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
↓
Add 50 µl of standard dilutions, samples, or positive control to each well. Add 50 µL of 1x Antibody solution to each well. Incubate 2 hours on the plate shaker at RT. Note: DO NOT WASH. PROCEED TO NEXT STEP.
↓
Add 50 µl 1x Biotin 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 all wells. 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 1-3 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.