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

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

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

PURCHASE INFORMATION:

ELISA NAME	RAT/MOUSE NGAL ELISA
Catalog No.	SK00233-06
Lot No.	
Formulation	96 T
Standard range	0.0128 - 40 ng/mL
Sensitivity	0.0128 ng/mL
Sample Volume	50 µl of diluted samples
Dilution Factor	4 (Optimal dilutions should be determined by each laboratory for each application with pretest)
Sample Type	Serum, EDTA plasma, Cell Culture Supernates
Specificity	Mouse and Rat NGAL
Intra-assay Precision	4%
Inter-assay Precision	8%
Storage	4°C

ORDER CONTACT:
AVISCERA BIOSCIENCE INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Mouse NGAL ELISA employs the quantitatively competitive enzyme immunoassay technique in which mouse NGAL present in samples competed with a fixed amount of biotinylated mouse NGAL for sites on purified rabbit IgG specific against mouse NGAL. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, 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 the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse NGAL bound in the initial step. The sample values are then read off the standard curve.

Mouse NGAL ELSA has been shown to accurately quantify the recombinant and natural mouse NGAL. Results obtained using natural mouse NGAL showed dose response curves that were parallel to the standard curves obtained using the kit standards.

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 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
R-Microplate – 96 well microplate precoated with polyclonal anti-rabbit IgG, one plate	**RM01**	**1 plate**
NGAL Standard – 1000 ng/vial of recombinant mouse NGAL in a buffered protein base with preservatives; lyophilized.	**233-06-01**	**1 vial**
Biotin Solution – 350 µL/vial, 10-fold Concentrate of mouse/rat NGAL biotinylated with preservatives; lyophilized.	**233-06-02**	**1 vial**
Antibody – 350µl/vial, 10-fold Concentrate of polyclonal purified IgG against mouse/rat NGAL with preservatives; lyophilized.	**233-06-03**	**1 vial**
Positive Control – one vial of recombinant mouse/rat NGAL , lyophilized (optional)	**233-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin-HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL/vial of buffered protein based solution with preservatives	**DB18**	**1 vial**
HRP Diluent Solution - 12 mL/vial of buffered protein based solution with preservatives	**DB06C**	**1 vial**
Wash Buffer - 50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 vial**
Substrate Solution – 11 ml/vial of TMB substrate solution	**TMB01**	**1 vial**
Stop Solution – 11 ml/vial of 0.5N HCl	**S-STOP**	**1 vial**
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 and Biotin Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Antibody Solution and Biotin Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold Concentrate and other kit 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 and seal along entire edge of zip-seal. Microplate may be stored for up to 6 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

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.

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

Mouse/Rat serum and plasma samples may need to be diluted 4-fold. A suggested 4-fold dilution is 30 µL sample + 90 µL Dilution Buffer**. Optimal dilutions should be determined by each laboratory for each application with pretest.**

Use polypropylene test tubes.

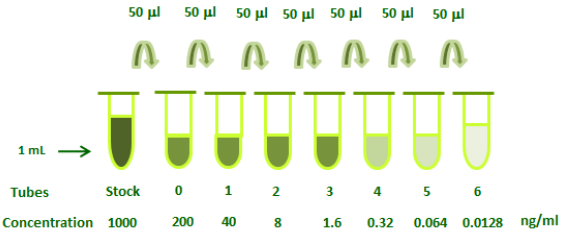
REAGENT PREPARATION

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 Wash Buffer.

Standard - Refer to vial label for reconstitution volume. 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 #0 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **40 ng/mL** standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	1000 ng/ml
# 0	50µl of stock	200µl	200 ng/ml
# 1	50µl of 0	200µl	40 ng/ml
# 2	50µl of 1	200µl	8 ng/ml
# 3	50µl of 2	200µl	1.6 ng/ml
# 4	50µl of 3	200µl	0.32 ng/ml
# 5	50µl of 4	200µl	0.064 ng/ml
# 6	50µl of 5	200µl	0.0128 ng/ml



Antibody Solution - Reconstitute the **Antibody Concentrate** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Antibody solution.

Biotin Solution – Reconstitute the **Biotin Concentrate** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Biotin solution.

Streptavidin-HRP Conjugate – Pipette 11.88 mL of **HRP Diluent Solution** (DB06C) 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.*

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

ASSAY PROCEDURE

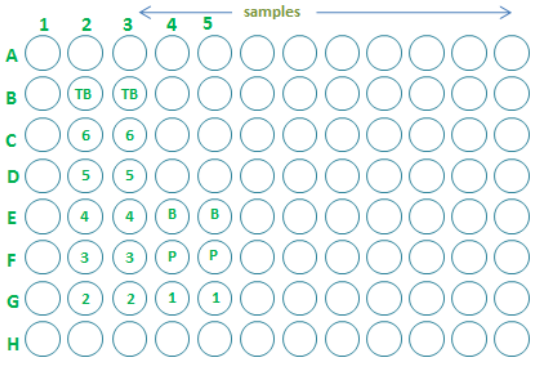
Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

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 and seal.
3. Leave well E4 and E5 as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTINYLATED SOLUTION INTO BLANK WELLS.***
4. Set B2 and B3 as total binding (TB). Add 50 µl per well of **Dilution Buffer**.
5. Add 50 µl per well of **Standard solution** from #6 to #1 (reverse order of serial dilution) to the appropriate wells (C2, C3 to G2, G3 and G4, G5). Add 50 µl per well of **Positive Control** into wells F4 and F5. Add 50 µl per well of **samples** into appropriate wells.
6. Add 25 µl per well of **1x Antibody Solution** into total binding, standard, PC and samples 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 25 µl per well of **1x Biotin Solution** into total binding, standard, PC and samples wells. Cover with plate sealer and incubate at room temperature for 2 hours on microplate shaker.
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 45 minutes at room temperature.
10. Aspirate and wash as step 8.
11. Add 100 µl of **Substrate Solution** to each well. Incubate for 12-18 minutes 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 micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, PC, 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.



TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0.058
TB	0.680
0.0128	0.619
0.064	0.638
0.32	0.520
1.6	0.288
8	0.046
40	0.009

*Lot No.:

** Positive Control: 10 – 21 ng/mL

CALIBRATION

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

SENSITIVITY

The minimum detectable dose (MDD) of mouse/rat NGAL 0.0128 ng/mL.

SUMMARY OF ASSAY PROCEDURE

Prepare reagents, samples and standards
↓
Add 50 µl of standard, samples, positive control to each well. Add 25 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 25 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 45 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 12-18 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min

SPECIFICITY

Mouse NGAL ELISA kit recognizes recombinant and endogenous Mouse NGAL. The data also indicated that rat serum samples were competitively bound to antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. That means rat serum samples cross-react with Mouse NGAL ELISA kit.

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