Mouse NGAL ELISA

by Exocell

Store this kit at 4°C upon receiving it.

Competitive Assay for the quantitative determination of mouse NGAL (catalog #1040)

MOUSE NGAL WORKFLOW

1. WARM KIT CONTENTS TO ROOM TEMPERATURE
2. DILUTE STANDARDS AND SAMPLES
3. ADD TO WELLS
4. ADD ANTI-MOUSE NGAL ANTIBODY
5. INCUBATE FOR 2 HOURS
6. WASH PLATE 3 TIMES WITH WASH BUFFER
7. ADD HRP-CONJUGATE ANTIBODY
8. INCUBATE FOR 60 MINUTES
9. WASH PLATE 5 TIMES WITH WASH BUFFER
10. ADD TMB, COLOR DEVELOP FOR 5-20 MINUTES
11. ADD COLOR STOPPER

READ 450 NM

RESULTS

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Intended Use: Ethos Mouse NGAL ELISA is designed to measure mouse NGAL in a competitive format in urine, serum or cell culture supernatant. It is for research purposes, and is not intended for diagnostic use.

Description: Ethos Mouse NGAL ELISA uses recombinant mouse NGAL as standard and an antibody raised against recombinant mouse NGAL. This antibody does not react with mouse albumin or mouse IgG. Mouse urine samples showed dose-response curves that were parallel to the standard curve indicating that this kit can be used to determine relative levels of native mouse NGAL. The assay is conducted in an indirect competitive mode and the results are reported as ng/ml mouse NGAL.

The Ethos Mouse NGAL ELISA plates are supplied coated, blocked, dried and ready to use. To complete the assay, the diluted standards and samples are added to respective wells. The anti-mouse NGAL is then added. This antibody interacts and binds with the NGAL in solution or with that immobilized to the plate, hence the notion of competitive binding. A subsequent reaction with HRP-conjugated second antibody then binds to the anti-mouse NGAL bound to the stationary phase plate. The bound conjugated antibody is then detected using a chromogenic reaction. Color intensity in Ethos Mouse NGAL is inversely proportional to the logarithm of the concentration of mouse NGAL in the fluid phase.

Kit Contents:

1. 1 96-well Mouse NGAL Assay Plate
2. 2 NGAL Reaction Buffer (or DILUENT)
3. 1 Mouse NGAL Standard
4. 1 Anti-Mouse NGAL Antibody
5. 1 HRP Conjugate Second Antibody
6. 1 TMB Color Developer
7. 1 Acid Color Stopper
8. 1 Instructions

All kit reagents are supplied in ready to use liquid form.

Note: Items 2 through 5 contain 0.05% Proclin 300 (active component Isothiazolin) as preservative.

Color Stopper contains 2.0 N Sulfuric Acid. Refer to the MSDS online at Ethos Biosciences' website for details (www.ethosbiosciences.com/msds).

Save and store at 4ºC all unused reagents and strips (in sealed pouch with included desiccant pack) for future use. Do not use this kit beyond its expiration date.

Other Materials required but not provided:

EIA Wash Buffer: 0.15 M NaCl, 0.01 M Triethanolamine, pH 6.8, 0.05% Tween 20, deionized water.

Microplate reader equipped to determine absorbance at 450 nm is required.

Adjustable pipettes and pipette tips to measure volumes ranging from 1 *u*l to 1000 *u*l.

Multi-channel pipettors capable of delivering 50 and 100 *u*L are recommended.

Microfuge tubes for dilution of standard and samples.

Wash bottle or automated microplate washer.

Specimen Collection and Storage: Samples should be clear and free of hemolysis.

Mouse urine: Collect urine samples without preservative. DO NOT CENTRIFUGE. Vortex the sample then freeze. Urine samples may be stored frozen at < -70°C for up to 2 months. *Avoid repeated freeze-thaw cycles.*

Mouse serum: Allow clotting of mouse blood for at least 30 minutes. Centrifuge for 10 minutes at 1000 x g. Recover serum layer. Assay immediately or store serum samples at -70ºC. *Avoid repeated freeze-thaw cycles.*

Cell Culture Supernatant/ Cell Lysate: Centrifuge all samples to remove debris prior to analysis. Assay immediately or store samples at -70ºC. *Avoid repeated freeze-thaw cycles.*

Limitations: It is the responsibility of the investigator to determine if the presence of experimental compounds or their metabolites in the urine may affect the assay results.

Gross hemolysis of the sample may affect assay results.

Gross microbiological contamination may affect assay results.

NGAL concentrations in urine and serum samples may vary in normal and diseased animals, by the collection method used, as well as by the mouse strain and sex. It is recommended to use the same mouse strain and sex when designing a research study. Samples should be run at multiple dilutions to determine the optimal dilution factor for accurate quantitation.

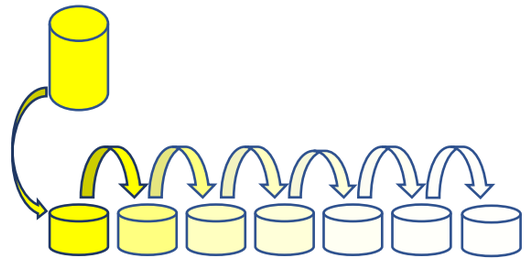
Assay Procedure: Allow reagents and samples to come to room temperature before running the assay. The assay performs better when room temperature is between 18ºC and 25˚C.

Standard and Samples, once at room temperature, should be gently mixed then allow any sediment to settle for 30 - 60 minutes. Alternatively, centrifuge the sample at 1000 x g for 1 minute. Use the supernatant in the assay.

These instructions are written with the intent to complete the assay using duplicate wells for each dilution of standard, and each dilution of sample. The Mouse NGAL ELISA may be used to determine the concentration of up to 40 samples if performed at a single dilution.

Standard Dilutions: The standard is supplied as a two-fold concentration. This procedure describes the preparation of seven (7) two-fold serial dilution of standard. Standards should be prepared fresh.

1. Prepare 7 microfuge tubes with 120 uL of NGAL Reaction Buffer per tube.
2. Label the tubes numbers 1-7.
3. Transfer 120 uL of Mouse NGAL Standard Stock to tube 1; this is a 1:2 dilution of the standard.
4. Mix contents by aspirating and expelling the fluids 5 times.
5. Transfer 120 uL of solution from tube 1 to tube 2.
6. Mix as before.
7. Continue this procedure through tube number 7.
8. Tubes 1-7 now contain dilutions representing 400, 200, 100, 50, 25, 12.5, 6.25 ng/ml Mouse NGAL. Further dilution to 3.12 ng/ml and running undilute standard is possible if needed to extend the standard curve.



Preparation of Sample Dilutions:

- The user needs to estimate the concentration of the NGAL in the sample and select the correct dilution factor.
- A starting dilution of 1:5 for mouse urine specimens is suggested. More concentrated samples may show urine effects but may be possible. For initial studies, and particularly if both normal and diseased samples are represented, it is wise to complete the analysis at more than one dilution, for example 1:5, 1:10, 1:20.
- A starting dilution of 1:50 for mouse serum specimens is suggested. Again, for initial studies, particularly when normal and diseased samples are represented, it is wise to complete the analysis at more than one dilution.

It is recommended that sample dilutions be performed in tubes; dilution in the plate is not recommended.

Addition of Controls, Standard NGAL Dilutions and Samples to the plate:

Label the strips of the plate with an indelible marker 1-12. This will allow reconstruction of the plate should strips fall out during the washing procedures.

The diluted standards and samples may be added directly to the dry plate. The plate design described here includes two controls: a negative control termed C0, and a positive one termed C1. These are placed in wells A1, and A2 respectively. All other wells receive either diluted standard or diluted sample. The standard and/or sample assay volume is 50 uL per well.

1. Add 100 *u*L NGAL Reaction Buffer from the stock bottle to well A1. This is the negative control "C0" and will be used to standardize or "blank" the microplate reader.
2. Add 50 *u*L NGAL Reaction Buffer to well A2. This is the positive control "C1" and is a qualitative indicator of assay performance.
3. With a fresh tip, transfer 50 *u*L aliquots of NGAL Standard Dilution Tube 1 (1:2 dilution of stock) to wells B1 and B2.
4. With a fresh tip, transfer 50 *u*L aliquots NGAL Standard Dilution Tube 2, and to wells C1 and C2.
5. Continue transferring diluted standard to the plate in this fashion, i.e. in order through H1 and H2, taking care to use a fresh tip for each new dilution.
6. Using a new tip, add 50 *u*L aliquots of Diluted Sample to wells A3 and A4.
7. Continue adding diluted samples to the plate, taking care to change the tip for each one.
8. The plate now contains controls, standard dilutions, and diluted experimental samples in duplicate for the balance of the plate.

Primary Antibody Incubation: Reaction with **Anti-Mouse NGAL Ab**.

1. **Do not add Anti-Mouse NGAL AB to well A1.** This is the C0 or negative control.
2. **After** adding standard and samples to all wells being used, add 50 *u*L of Anti-Mouse NGAL Ab to A2 and to all remaining wells. Use of a 50 *u*L multichannel pipettor is recommended.
3. Cover and incubate the plate in a moist chamber at room temperature (approximately 18-25°C) for 2 hours.

Secondary Antibody Incubation: Reaction **with anti-IgG-HRP Conjugate**:

1. Wash the plate for 3 cycles using a squirt bottle or auto-plate washer. Each cycle consists of: filling each well with 300 ul of EIA Wash Buffer and then aspirating each well. Complete removal of liquid after each cycle is essential for good performance. After the last cycle invert the plate on a clean paper towel and tap gently to blot any adherent fluids. Do not allow plate to dry out.
2. Add 100 *u*L of anti-IgG-HRP Conjugate to every well on the plate. Use of a 100 *u*L multichannel pipettor is recommended.
3. Cover and incubate the plate for 60 minutes at room temperature in a moist chamber.

Color Development:

1. Wash Plate as Step 1, above, for 5 wash cycles.
2. Add 100 *u*L of Color Developer to each well. The wells will turn blue with the intensity inversely proportional to the concentration of NGAL in the well.
3. Develop 5- 20 minutes.
4. Add 100 *u*L of Color Stopper to each well. The color will change from blue to yellow.
5. Use a plate reader to determine and record the absorbance of all experimental wells at 450 nm, blanked against well A1. If the reader is capable of reading at 630 nm, the absorbance reading at 630 nm may be subtracted from the 450 nm reading.

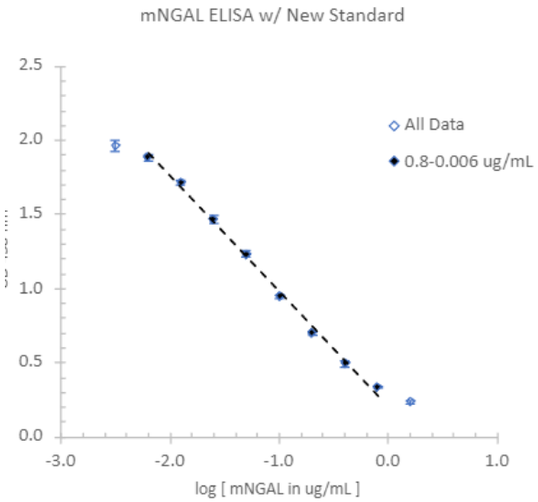
Analysis:

The results are analyzed using a computer-based curve-fitting software program for a 4- parameter logistics curve fitting algorithm plotting the mean absorbance (y axis) against the protein concentration (x axis). The NGAL concentration of the samples can then be interpolated from the standard curve. Multiply the concentration by the dilution factor of each undilute sample.

Alternatively, if a 4 PL fit program is not available, prepare a spreadsheet entering appropriate data including standard dilution, concentration, sample dilution and absorbance data. Determine the mean for replicate wells.

Prepare a semi-logarithmic plot of standard dilutions with the log [NGAL] on the x-axis and the mean absorbance on the y-axis.

The data that fall into the linear portion of the dose-response curve constitute the usable portion of the assay.



Subject these data to semi-logarithmic analysis to yield a mathematical model, of the form:

$$\log_{10} [\ NGAL\ ] = m\ A_{450} + b$$

NGAL concentration is determined by taking the anti-log of the calculated values from this equation.
Multiply by the dilution factor of the sample to determine the concentration of undilute sample.

Quality Control:

- Record Keeping: It is good laboratory practice to record the lot numbers and dates of the kit components and reagents for each assay.
- Sample Handling: The samples should be secured, processed and stored as discussed above.
- Dilute Standard and Samples carefully. For each standard and sample, a fresh tip should be used.
- Template: Record the position of each standard or sample on a microplate template.

Trouble Shooting:

1. No color appears after adding Color Developer: One or more reagents may not have been added. Repeat assay. Be sure to store the kit appropriately. Do not use expired kits or reagents from other kits.
2. Color in wells too light: Longer incubation with Color Developer may be required. If the color is still too light after 20 minutes development, repeat the assay but increase the secondary incubation to 2 hours.
3. Color in wells is too dark: Be sure to wash with the appropriate buffer. Be sure to blot dry after washing. Do not allow wells to dry out. Ensure that the standards and samples were added to the plate first followed by the primary antibody. Decrease the development time. If a 5-minute development yields the C1 too dark with no appropriate dose-response of the standard curve, repeat the assay and reduce the secondary incubation to 45 minutes.

   If color is dark and the standard dilutions fail to show the appropriate dose-response, Color Developer may have been contaminated with conjugate or the plate was poorly washed. The color developer should be clear to a vary pale blue at room temperature. Be sure that all reagents, standards and samples are at room temperature throughout the assay. Repeat the assay and take care in the pipetting and in the washing operations.
4. Color in sample well(s) is darker or lighter than lowest or highest concentrations of the standard curve. Change sample dilution appropriately.
5. Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay.
6. Microplate ELISAs may be prone to edge effects (or prozone effect) wherein the outer rows and columns show a darker response than the inner ones. This effect may be minimized by incubating the plate in a closed humid container. A plastic food storage container with a tight-fitting lid containing a water moistened paper towel work well in this respect. Place the moistened towel in the bottom of the container and place the microplate upon it; add enough room temperature water to barely touch the bottom of the wells. Position the cover and incubate as described. When removing the plate, be sure to gently blot the bottom of the wells to remove excess water before continuing with the wash cycles and assay steps.

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