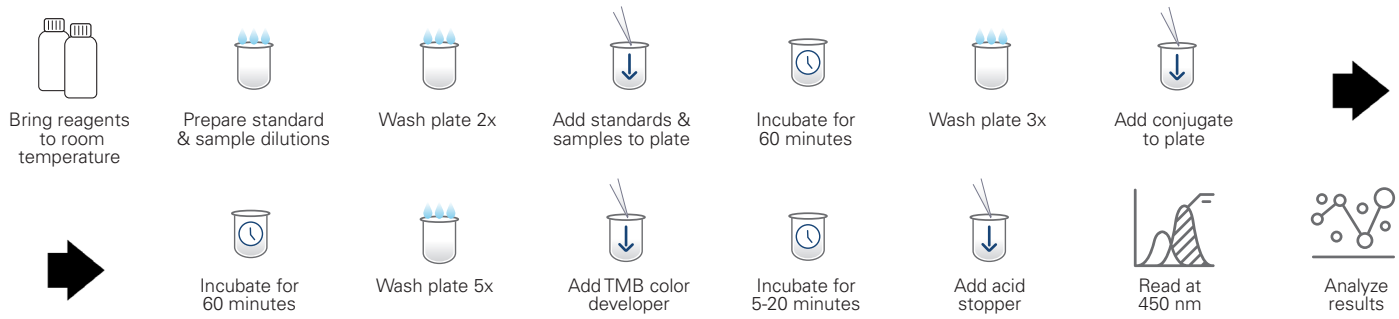
Exocell™

HUMAN NGAL ELISA



DESCRIPTION

Ethos Biosciences' Human NGAL ELISA is designed to measure human NGAL in biological samples in a direct, antibody sandwich format. It is for research purposes, and is not intended for diagnostic use.

Ethos' Human NGAL ELISA uses recombinant human NGAL as standard and two antibodies raised against recombinant human NGAL. The antibodies do not react with human albumin or human IgG. Human 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 human NGAL. The assay results are reported as ng/ml human NGAL.

The Ethos Human NGAL ELISA plate is supplied coated, blocked, dried and ready to use. To complete the assay, the plate is washed 2 times and diluted standards and samples are added to respective wells and the plate incubated. After washing, the anti-human NGAL-HRP conjugate is added. This antibody interacts and binds with the NGAL bound to the antibody coated on the plate. The bound conjugated antibody is then detected using a chromogenic reaction. Color intensity in Ethos' Human NGAL ELISA is proportional to the concentration of human NGAL in the sample.

KIT CONTENTS

- 1 x 96-well Human NGAL Assay Plate
- 2 x NHE-BSA Diluent
- 1 x Human NGAL Standard
- 1 x Anti-Human NGAL-HRP Conjugate
- 1 x TMB Color Developer
- 1 x Acid Color Stopper
- 1 x Instructions

All kit reagents are supplied in ready to use liquid form.
Note: NHE-BSA Diluent, Human NGAL Standard, & Anti-Human NGAL-HRP Conjugate all contain 0.05% Proclin 300 (active component Isothiazolinone) 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 4°C 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 pipettors and pipette tips to measure volumes ranging from 1 ul to 1000 ul.

Multi-channel pipettor capable of delivering 100 uL is 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.
Human 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.
Human serum: Allow clotting of human 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.
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 20ºC 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 Human 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 concentrate. 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 225 uL of NHE-BSA Diluent per tube.
2. Label the tubes numbers 1-7.
3. Transfer 225 uL of Human 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 225 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 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.20 ng/ml human NGAL. Undilute standard (25 ng/ml) 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 human urine specimens is suggested. More concentrated samples may show urine effects but may be acceptable. 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 human 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.

Wash the plate for 2 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.

The plate design described here includes a negative control termed C0. This placed in wells A1, and A2. All other wells receive either diluted standard or diluted sample. The standard and/or sample assay volume is 100 uL per well.

1. Add 100 uL NHE-BSA Diluent from the stock bottle to wells A1 and A2. This is the negative control "C0" and will be used to standardize or "blank" the microplate reader.
2. With a fresh tip, transfer 100 uL aliquots of NGAL Standard Dilution Tube 1 (1:2 dilution of stock) to wells B1 and B2.
3. With a fresh tip, transfer 100 uL aliquots NGAL Standard Dilution Tube 2, and to wells C1 and C2.
4. 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.
5. Using a new tip, add 100 uL aliquots of diluted sample to wells A3 and A4.
6. Continue adding diluted samples to the plate, taking care to change the tip for each one.
7. The plate now contains controls, standard dilutions, and diluted experimental samples in duplicate for the balance of the plate.

Primary Antibody Incubation: Reaction with capture anti-Human NGAL antibody on the plate.

- After adding standard and samples to all wells being used, incubate the plate in a moist chamber with a lid at room temperature (approximately 20-25˚C) for 1 hour.

Secondary Antibody Incubation: Reaction with detector anti-

Human NGAL-HRP antibody Conjugate:
1. Wash the plate for 3 cycles, as described above.
2. Add 100 uL of anti-human NGAL-HRP Conjugate to every well on the plate. Use of a 100 uL multichannel pipettor is recommended.
3. Cover and incubate the plate for 60 minutes at room temperature in a moist chamber.

Color Development
1. Wash the plate for 5 wash cycles, as described above.
2. Add 100 uL of Color Developer to each well. The wells will turn blue with the intensity directly proportional to the concentration of NGAL in the well.
3. Develop 5- 20 minutes.
4. Add 100 uL 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 or 5 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 to determine the concentration of the 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 plot of standard dilutions with the NGAL concentration (ng/ml) 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 linear regression analysis to yield a mathematical model, of the form:

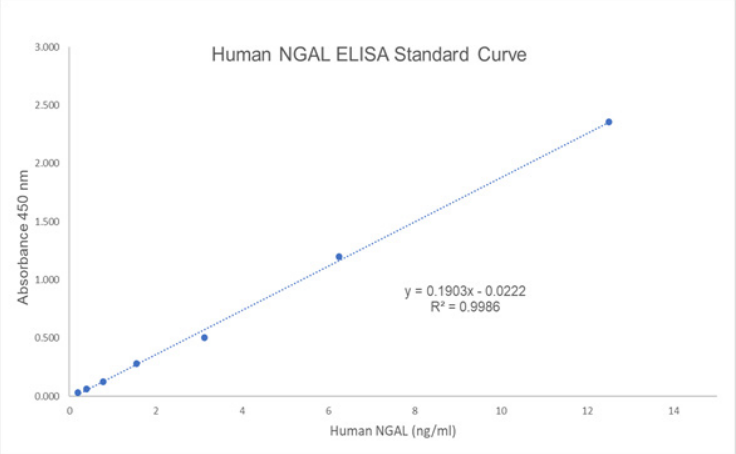
$$Abs = m\,[NGAL] + b$$

which rearranges into:

$$[NGAL] = (A450 - b) / m$$

NGAL concentration of an undilute sample is determined by taking the calculated values from this equation and multiplying by the dilution factor.

See standard curve in next column.



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

- 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.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 1.5 hours.
- 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 very 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.
- Color in sample well(s) is lighter or darker than lowest or highest concentrations of the standard curve. Change sample dilution appropriately.

(Troubleshooting continued on the next page)

- Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay.
- Microplate ELISAs may be prone to edge effects 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.

Human NGAL ELISA v 2.0: July, 2022

PRODUCT INFORMATION

Cat. #	Description
1038	Human NGAL ELISA

Order today at **EthosBiosciences.com**