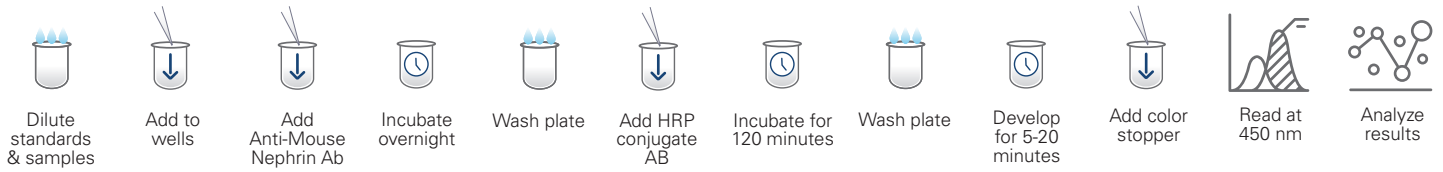
MOUSE NEPHRIN ELISA



DESCRIPTION

Our Mouse Nephrin ELISA is a competitive ELISA for the measurement of nephrin in human urine specimens. It is intended for research purposes, but is not for diagnostic applications.

Technical Background: Nephrin is a transmembrane protein expressed in podocytes, the visceral epithelial cells that line the outer aspect of the glomerular basement membrane (1-5). These cells have interdigitating foot processes constituting slit diaphragms that regulate the passage of plasma proteins across the glomerular barrier, therefore performing a filtration function. Mutation of the nephrin gene, altered nephrin production, and abnormal podocyte function all lead to proteinuria in diseases affecting the glomerulus such as nephrotic syndrome and diabetes. (1,6-8). Kidney injury may be accompanied by release of nephrin and/or shedding of podocytes into the urine. Studies in animals and diabetic humans support the notion that nephrinuria is a marker for, and may reflect severity of, glomerular damage in kidney injury and disease (9-11). Measurement of urinary nephrin may enable early detection of renal dysfunction and/or assessment of renal response to therapeutic interventions.

Mouse Nephrin ELISA uses mouse nephrin as a standard (Mouse Nephrin Standard), and an antibody raised against the N-terminal portion of mouse nephrin (Anti-Mouse Nephrin Ab). This antibody does not cross-react with mouse albumin.

Mouse Nephrin ELISA Is an indirect competitive assay. The plates are coated with a preparation of nephrin. Diluted standard and samples are added to respective wells.

The anti-mouse nephrin antibody is added, which binds with either the nephrin immobilized to the stationary phase or with that in the fluid phase; hence the notion of competitive binding.

Unbound reactants are washed away, and reaction with HRP-Conjugate Ab binds to antibody bound to the solid phase antigen labeling it with enzyme.

After washing, a substrate and indicator are added, and a chromogenic enzymatic reaction ensues. The reaction is stopped after a short incubation period with the addition of acid. The color intensity in Mouse Nephrin ELISA is inversely proportional to the logarithm of mouse nephrin concentration in the initial fluid phase.

SPECIMEN COLLECTION AND STORAGE

Collect urine samples without preservative. Freeze and store at -60˚C for up to 2 months. Prior to assay, allow the samples to come to room temperature. Do not apply heat to thaw frozen samples.

KIT CONTENTS

- 1 Mouse Nephrin Assay Plate
- 2 EIA Diluent
- 1 Mouse Nephrin Standard
- 1 Anti-Mouse Nephrin Ab (Antibody)
- 1 HRP Conjugate Ab (Antibody)
- 1 TMB Color Developer
- 1 Acid Color Stopper
- 1 Instructions

Mouse Nephrin Assay Plates are precoated and ready to use. All kit reagents are supplied in ready to use liquid form.

Materials and Equipment Needed, but Not Included in Kit

- Wash Buffer: Ethos Biosciences EIA Wash Buffer works well. PBS with 0.05% Tween 20 is also satisfactory. Water is unsatisfactory.
- Micropipettors and Multi-channel pipettors and tips
- Tubes for dilution (Microfuge tubes work well).
- Microplate reader equipped to measure absorbance at 450 nm is required.

LIMITATIONS

It is the responsibility of investigators to determine if Mouse Nephrin ELISA is suitable for use with their samples. The presence of experimental compounds or their metabolites in the urine and gross microbiological contamination may affect assay results.

ASSAY PROCEDURE

Allow reagents to come to room temperature before running the assay.

Standard and Samples should be mixed gently then allowed to sit for a minimum of 15 minutes to allow particulates to settle.

These instructions are written with the intent to complete the assay using duplicate wells for each dilution of standard, and each dilution of sample. This design allows inclusion of controls, standard curve and provides for analysis of up to 40 samples.

Standard Dilutions: This procedure describes the preparation of seven (7) two-fold dilutions of standard.

1. Label the tubes numbers 1-7.
2. Add 120 uL of EIA DILUENT to each tube.
3. Transfer 120 uL of Mouse Nephrin Standard to tube 1.
4. Mix contents by aspirating and expelling the fluids 5 times.
5. Transfer 120 uL of from tube 1 to tube 2.
6. Mix as before.
7. Continue doing this through tube number 7.
8. Tubes 1-7 now contain dilutions representing 2.0- 0.0313 ug/ml mouse nephrin.

Preparation of Urine Sample Dilutions: Nephrin concentrations in urine from normal and diseased animals vary, and collection methods affect concentration. A starting dilution of 1:5 for specimens is suggested, but it is wise to complete the analysis at more than one dilution. It is strongly recommended that sample dilutions be performed in tubes, and not attempted in the plate.

Addition of Controls, Standard Nephrin Dilutions and Samples to the plate: Label the strips of the plate with an indelible marker 1-12. This will allow reassembly 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 uL EIA DILUENT to well A1. This is the negative control "C0" and will be used to standardize or "blank" the microplate reader.
2. Add 50 uL EIA DILUENT to well A2. This is the positive control "C1" and is a qualitative indicator of assay performance.
3. With a fresh tip, pre-wet the tip with Nephrin Standard dilution 1, and transfer 50 uL aliquots to wells B1 and B2.
4. With a fresh tip, pre-wet the tip with Dilution 2, and transfer 50 uL aliquots 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 pre-wet the tip with the new dilution each time.
6. Using a new tip, pre-wet the tip with diluted sample, and add 50 uL aliquots of Diluted Sample to wells A3 and A4.
7. Continue adding diluted samples to the plate, taking care to change and pre-wet the tip for each one.
8. The plate now contains controls, standard dilutions, and diluted experimental samples in duplicate in the balance of the plate.

Primary Incubation: Reaction with Anti-Mouse Nephrin Ab

1. Do not add Anti-Mouse Nephrin Ab to well A1.
2. Add 50 uL of Anti-Mouse Nephrin Ab to A2 and to all remaining wells.
3. Incubate the plate overnight at room temperature in a moist chamber. A plastic food storage container works well. Place a water moistened paper towel in the bottom of the container, place the plate upon it and position the cover. Incubate as described.

Secondary Incubation: Reaction with HRP -Conjugate Ab:

1. Wash the plate five times with Wash Buffer.
2. Flip out the fluids, and blot dry after the final wash.
3. Add 100 uL of HRP-Conjugate Ab to each well.
4. Incubate the plate 2 hours at room temperature in a moist chamber.

Color Development

1. Wash Plate 5X as described above.
2. Flip out and blot after final wash.
3. Add 100 uL of Color Developer to each well.
4. Develop 5- 20 minutes at room temperature.
5. Add 100 uL of Color Stopper to each well.
6. Determine absorbance at 450 nm blanked against well A1.

Analysis: This analysis uses spreadsheet software such as Microsoft Excel.
Prepare a spreadsheet entering a row for each sample, and a column for each for: Sample ID, dilution, standard concentration, log[std concentration], replicate absorbance data, mean absorbance, est Concentration and undilute concentration.
Enter standard/sample information and absorbance data, and determine the mean absorbance for replicate wells.
Prepare a semi-logarithmic plot of standard dilutions with the log [Nephrin] 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, log concentration and absorbance to regression analysis to yield a model of the form:

log10 [Nephrin] = m A450 + b

Nephrin 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. For assistance with analysis, please contact customer service.

Quality Control: It is good laboratory practice to record lot numbers and expiration dates of the kit components for each assay.

Sample Handling: The samples should be secured, processed, and stored as discussed above. Dilute Standard and Samples carefully. For the standards, a single tip may be used to prepare the dilution series. For experimental samples a fresh tip should be used for each urine specimen.

TROUBLE SHOOTING

- "No color appears after adding Color Developer" One or more additions may have been omitted or reagents compromised by improper storage. Repeat assay.
- "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 double the secondary incubation time.
- "Color in wells is too dark" Decrease the development time. If a 5-minute development is still too dark; reduce secondary incubation by half.
- "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. Repeat the assay and take care in the pipetting and in the washing operations.
- "Color in sample well(s) is darker or lighter than lowest or highest concentrations of the standard curve" All samples need to fall within the standard curve to give valid quantitative measurement data. If a sample is darker than the darkest standard, it is too dilute. If it is lighter than the lightest standard it is too concentrated. Repeat assay and dilute appropriately.
- "Poor agreement between duplicate wells" There is most likely pipetting error. Repeat the assay and ensure precise pipetting and proper dilution.
- Hint: Microplate ELISAs may be prone to edge effects wherein the wells show a darker response than the inner ones due to evaporation. This effect may be minimized by incubating the plate in a closed humid chamber. A plastic or glass container with sealable lid works well. Place a water moistened paper towel in the bottom of the container, and place the plate upon it. Close the cover and incubate as described.
- Hint: In a competitive ELISA antibody binding occurs with both fluid borne and surface bound antigen. In dilute samples little antigen is present in the fluid phase, and most of the antibody binds to the surface bound antigen. This leads to a dark color upon development. In contrast, at high antigen concentrations, most of the antibody binds to antigen in the mobile phase; leaving little to bind to the antigen on the solid phase. Subsequent reactions and color development produce lighter color. Therefore; color intensity in a competitive ELISA is inversely proportional to the log of antigen concentration.
- Hint: There is variability of urinary nephrin due to animal, treatment and collection protocol. Nephrin measurements may be normalized using creatinine concentration measured in the same sample.

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PRODUCT INFORMATION

Cat. #	Description
1037	Mouse Nephrin ELISA

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