mdbioproducts.

MonELISA® Series
Uromodulin (THP) ELISA

Catalog Number M036020

For the quantitative determination of Uromodulin (Tamm-Horsfall Glycoprotein, THP, UMOD) in urine and serum samples.

For research use only.

INTRODUCTION

Uromodulin glycoprotein (also known as Tamm-Horsfall Glycoprotein, THP) is the most abundant protein found in the urine of mammals (2, 4). Uromodulin is 616 amino acids in length with a molecular weight of 80 kDa, and is synthesized in the thick ascending loop of Henle (3, 4). While the specific function of Uromodulin is unknown, it appears to have a role in the regulation of salt and water excretion by the kidney (3). Soluble Uromodulin has been found to help protect against urinary tract infections of *E. coli* and *P. mirabilis* by inhibiting the binding of the bacteria to the epithelial cells of the urinary tract (1, 6, 8). In addition, it has been suggested that Uromodulin may help prevent renal stone formation (7). Defects in Uromodulin are associated with human diseases such as familial juvenile hyperuricemic nephropathy (FJHN) and medullary cystic kidney disease (MCKD2) (4, 5). In serum, Uromodulin levels have been associated with kidney disease (10), graft failure in renal transplant recipients (11), and cardiovascular disease (12, 13).

PRINCIPLE OF THE ASSAY

The MonELISA® Uromodulin ELISA is a colorimetric based sandwich immunoassay utilizing a polyclonal antibody to Uromodulin bound on the surface of microwells as the capture antibody and a biotinylated polyclonal antibody to Uromodulin as the detection antibody. When antigen is added to the well it is bound by the immobilized antibody. The biotinylated antibody then binds to the antigen forming an antigen-antibody complex. Streptavidin-Peroxidase in the presence of an enzyme substrate quantifies analyte bound. Color development is directly proportional to the antigen concentration in the sample.

KIT COMPONENTS

Uromodulin Glycoprotein Microplate (Part PL30172) - The plate contains 12 x 8 strips coated with polyclonal antibody to Uromodulin Glycoprotein. The strips are ready to use.

Uromodulin Glycoprotein Standard (Part ST30171) - 1 vial (2500 ng/mL) of Uromodulin Glycoprotein (THP) in a protein buffer.

Conjugate Concentrate (Part BT30170) - 1 vial of a 100-fold concentrated biotinylated anti-Uromodulin Glycoprotein antibody in a stabilizing buffer diluted in buffer provided in Ancillary Reagent Kit, Catalog Number M036080.

HRP Streptavidin Concentrate (Part HP30006)- - 1 vial of a 100-fold concentrated streptavidin-HRP diluted in buffer provided in Ancillary Reagent Kit, Catalog Number M036080

Plate Sealer - 2 adhesive strips.

THESE ASSAY COMPONENTS ARE TO BE USED WITH

5-PLATE ANCILLARY REAGENT KIT, CATALOG NUMBER M036080

SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips
- Measuring cylinders
- Distilled or deionized water
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm

PRECAUTIONS

Stop Solution consists of diluted sulfuric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

The Assay Diluent contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

CRITICAL PARAMETERS

- Allow samples and all reagents to equilibrate to room temperature (22 - 25 °C) prior to performing the assay. This is especially important for the TMB Substrate.
- Adhere to recommended incubation temperatures in the protocol as variations may cause inconsistent or poor assay results.
- It is essential that all wells are washed thoroughly and uniformly. When washing is done manually, ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge these reagents before use.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the plate sealer except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

SAMPLE PREPARATION

Sample collection and storage:

Urine samples should be aseptically collected in mid stream and directly into a sterile container. It is recommended that if the samples can not be used immediately, the undiluted samples should be stored at -20 ºC. Aliquots are recommended to avoid repeated freeze-thaw cycles.

For serum samples, collect whole blood without anticoagulant and allow blood to clot between 2-8 ºC, if possible. Then the serum should be promptly separated, preferably in a refrigerated centrifuge. If the samples can not be used immediately, the undiluted samples should be stored at 2-8 ºC for up to 24 hours, or for up to 1 year at -20ºC or lower. Aliquots are recommended to avoid repeated freeze-thaw cycles.

Sample preparation:

The availability of Uromodulin in the urine sample may be affected by pH and the ionic strength of the urine sample. Published data have shown that correct pH and ionic conditions are required to avoid loss of the analyte through aggregation and subsequent sedimentation. The MD-1 Assay Diluent provided in this kit is designed to keep the Uromodulin in soluble form providing better linearity of the sample results. Other recommended buffers include the TEA (Triton/EDTA/Alkaline) Buffer containing 0.5% Triton x-100, and 20 mM EDTA at a pH of 7.5 (9). To remove particulate matter from the urine, the samples after dilution with MD-1 Assay Diluent or TEA Buffer can be centrifuged at low speed at 400x g for 5 minutes.

Sample Dilution:

Uromodulin levels in urine and serum may be highly variable and appropriate dilutions must be determined by each individual lab.

For human urine samples, a suggested starting dilution of 1:200 is recommended.

For canine or murine urine samples, a suggested starting dilution of 1:100 is recommended.

For serum samples, a suggested starting dilution of 1:2 is recommended.

REAGENT PREPARATION

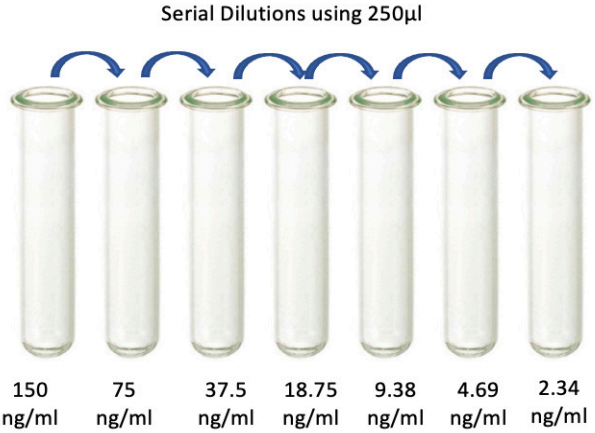
Bring all reagents to room temperature (22 - 25 °C) before use. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge before use. All diluents required are provided in the Ancillary Reagent Kit Catalog number M036080.

Wash Buffer (1X) - 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 450 mL of deionized water to prepare 500 mL of Wash Buffer (1X). Store for up to 30 days at 2 - 8 °C.

Conjugate (1X) - Dilute 60 µL of Conjugate Concentrate into 5.94 mL of Conjugate diluent. Discard after use.

Streptavidin-HRP (1X) - **Prepare within 30 minutes of use and keep protected from light**. Add 60 µL Streptavidin-HRP Concentrate to 5.94 mL of HRP Diluent. Prepare fresh Streptavidin-HRP (1X) for each assay. If running less than a full plate, prepare only the amount needed.

Standards - Label 7 standard tubes as shown below. Pipette 470 µL Assay Diluent into the 150 ng/mL standard tube and 250 µL Assay Diluent into the remaining tubes. Add 30µL the 2500 ng/mL standard to the 150 ng/ml tube then begin to produce a 2-fold dilution series (see below). The 150 ng/mL standard serves as the high standard and Assay Diluent serves as the zero (0 ng/mL) standard.



ASSAY PROTOCOL

It is recommended that all standards and samples be assayed in duplicate. Reagents can easily be trapped in the caps of small microfuge tubes. It is recommended to briefly centrifuge before use.

Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding.

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microplate strips from the plate frame and return them to the foil pouch containing the desiccant pack.
3. Add 50 µL of Standard or diluted sample in duplicate to each well. Cover with the plate sealer provided and incubate for 1 hour at 37 °C.
4. Aspirate and wash the wells 6 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
5. Add 50 µL Conjugate (1X) to each well. Cover with the plate sealer provided and incubate for 1 hour at 37 °C.
6. Aspirate and wash the wells 6 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
7. Add 50 µL of diluted Streptavidin-HRP to each well. Incubate for 30 minutes at 37 °C. **Protect from light.**
8. Aspirate and wash the wells 6 times with 200 µL per well of Wash Buffer (1X). Take care that all wells are filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.
9. Add 50 µL Substrate to each well and incubate for 10 minutes at 37 °C. **Protect from light.**
10. Stop the reaction by adding 50 µL of Stop Solution to each well. Gently tap the side of the plate to ensure thorough mixing.
11. Read the plate at 450 nm.

SUMMARY

Prepare reagents and samples as previously described.

Pipette 50 µL Standard or diluted sample in duplicate to each well.
Cover with plate sealer and incubate 1 hr. at 37 °C.

Aspirate and wash 6 times.

Add 50 µL of Conjugate (1X) to each well.
Cover with plate sealer and incubate 1 hr. at 37 °C.

Aspirate and wash 6 times.

Add 50 µL of diluted Streptavidin-HRP to each well. Incubate 30 min. at 37 °C. Protect from Light.

Aspirate and wash 6 times.

Add 50 µL of Substrate to each well. Incubate 10 min. at 37 °C. Protect from Light.

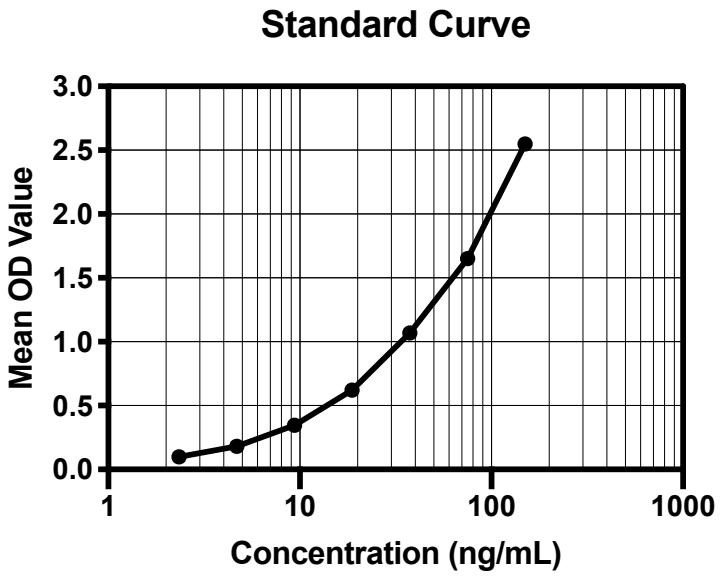
Add 50 µL of Stop Solution to each well. Read at 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using 4-parameter logistic (4-PL) curve fit.

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

Range: 2.3 ng/ml - 150 ng/ml



PERFORMANCE CHARACTERISTICS

Sensitivity

Sensitivity is defined as the minimal detectable dose determined by adding two standard deviations of the mean optical density value for twenty replicates of the zero standard and calculating the corresponding concentration. The sensitivity of the MonELISA® Uromodulin is typically less than 1 ng/mL.

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 15-20 times on one plate.

Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 15 successive assays with multiple users.

	Intra-assay Precision			Inter-assay Precision		
Control	1	2	3	1	2	3
Mean (ng/mL)	23.7	47.9	101.6	23	46.7	94.5
Standard Deviation	2.03	3.85	8.91	2.03	3.84	12.23
CV (%)	8.5	8.0	8.8	8.8	8.2	12.9

LINEARITY

Urine samples were diluted 1:200 and then serially diluted 1:2 in Assay Diluent. The mean linearity for all samples is 107% (range 94 - 144%).

Serum samples were serially diluted 1:2 in Assay Diluent. The mean linearity for all samples is 93% (range 60 - 104%).

CROSS-REACTIVITY

MonELISA® Uromodulin detects Uromodulin Glycoprotein levels in human, murine and canine urine and serum samples. Cross-reactivity with other mammalian urine samples has not been tested.

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TROUBLESHOOTING

Problem	Recommendation
Low Absorbance	· Check reagents for proper storage. · Check expiration date. · Check preparation of reagents. · Check incubation times and temperature. · Check reader wavelength.
High Absorbance/high zero standard value	· Check preparation of reagents. · Check incubation times and temperature. · Equilibrate ELISA reagents to room temperature (22 - 25 °C). · Ensure that every well of the ELISA plate is filled completely and emptied at every wash step. · Check that plates are blotted on tissue paper after washing.
Flat curve/poor reproducibility	· Check reagents for proper storage. · Check expiration date. · Check preparation of working standards. · Check incubation times and temperatures. · Use separate reservoirs for pipetting different solutions with multichannelled pipettes. Always use new pipette tips. · Check pipette calibration. · Ensure efficient washing procedure.

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