

## URINARY UROMODULIN / TAMM-HORSFALL URINARY GLYCOPROTEIN HUMAN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF  
HUMAN UROMODULIN CONCENTRATIONS IN  
URINE, SERUM AND PLASMA



ALWAYS REFER TO LOT SPECIFIC PROTOCOL  
PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ BEFORE USING THIS PRODUCT.

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

### PRODUCT INFORMATION:

**THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	URINARY UROMODULIN (UMOD) HUMAN ELISA KIT
Catalog No.	SK00927-08
Formulation	96 T
Lot No.	
Standard range	1.95- 500 ng/ml
Sensitivity	200 pg/ml
Sample Volume	100 µl
Dilution Factor	<b>100- 400 for urine (Optimal dilutions should be determined by each laboratory for each application)</b>
Sample Type	Urine, Serum, EDTA Plasma
Specificity	Human Urine Uromodulin
Calibration	Human Urine Uromodulin
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 8%
Storage	2 – 8° C for 10 months.
This kit contains sufficient materials to run 40 samples duplicated provided that assay is run according to protocol.	

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## DESCRIPTION

This Human Urinary Uromodulin (UMOD) Tamm-Horsfall Urinary Glycoprotein (THGP) ELISA Kit contains the necessary components required for the quantitative measurement of natural human uromodulin from urine, plasma, serum in a sandwich ELISA format.

This immunoassay contains human urine uromodulin protein and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify natural uromodulin samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human urine uromodulin. The capture antibody can bind to the human urine uromodulin in the standard and samples. After washing the plate of any unbound substances, a detection antibody against human urine uromodulin is added to the wells. After another washing of the plate, the Secondary Antibody-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human uromodulin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

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

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix 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.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>UMOD Microplate</b> – 96 well microplate coated with an antibody specific for human uromodulin.	<b>927-018-01</b>	<b>1 plate</b>
<b>UMOD Standard</b> – refer to lot specific of lyophilized human uromodulin.	<b>927-018-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 1.2 mL/vial of 10-fold concentrate of lyophilized antibody against human uromodulin.	<b>927-08-03</b>	<b>1 vial</b>
<b>Secondary Antibody-HRP Conjugate</b> – 120 µL/vial of 100-fold concentrated solution of Secondary Antibody-HRP Conjugate.	<b>SAMHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 45 mL of buffered solution with preservative.	<b>DB10</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> – 12 mL of buffered solution with preservative.	<b>DB08B</b>	<b>1 bottle</b>
<b>Wash Buffer</b> – 50 mL of 10-fold concentrated buffered surfactant with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>Substrate Solution</b> – 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

## STORAGE

**Unopened Kit:** Store at 2 – 8° C for up to 10 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C.

For long term Storage for Dilution buffer (**DB10**), store in -20° C.

Secondary Antibody-HRP Conjugate and Substrate Solution should be stored only at 2 ~ 8 °C.

Do not use kit past expiration date.

## ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at **620 ~ 650 nm**.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

### PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains detergents SDS solution. Wash immediately with water in case of contact on skin or eyes.

### SAMPLE COLLECTION AND STORAGE

**Urine** – Collect the second morning urine samples (mid-stream) in a sterile container and processed within 2 hours. Assay samples may use freshly collected and non-centrifuged urine samples. All urine samples need vortex for 10 s prior to dilute and test. Assay samples immediately or aliquot and store at  $\leq -70^{\circ}\text{C}$  for 4 months. Avoid repeated freeze-thaw cycles.

**Serum** – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at  $1000 \times g$  for 15 minutes and collect serum. Assay samples immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Plasma** – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at  $1000 \times g$  for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.**

### SAMPLE PREPARATION

Urine samples may require 100 ~ 400-fold dilutions using Dilution Buffer DB10 or ultrapure deionized water.

Serum or plasma samples may not require dilution.

**Optimal dilutions should be determined by each laboratory for each application with a 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 bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

**Human Uromodulin Standard** - Reconstitute the Uromodulin standard with refer to lot of **Dilution Buffer (DB10)**. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450  $\mu\text{L}$  of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **500 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	Refer to lot	500 ng/mL
# 1	150 $\mu\text{L}$ of stock	450 $\mu\text{L}$	125 ng/mL
# 2	150 $\mu\text{L}$ of 1	450 $\mu\text{L}$	31.25 ng/mL
# 3	150 $\mu\text{L}$ of 2	450 $\mu\text{L}$	7.8 ng/mL
# 4	150 $\mu\text{L}$ of 3	450 $\mu\text{L}$	1.95 ng/mL

**Detection Antibody** - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Dilution Buffer DB10** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Dilution Buffer DB10** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

**Secondary Antibody-HRP Conjugate** - Pipette 10.89 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 110  $\mu\text{L}$  of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of **Secondary Antibody-HRP Conjugate** should be used within a few hours (**protect from light**). **DO NOT FREEZE.**

## ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100  $\mu$ L of **Dilution Buffer (DB10)** to Blank wells.
4. Add 100  $\mu$ L of **Standard dilutions from #4-S or samples** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300  $\mu$ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100  $\mu$ L of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100  $\mu$ L of **Secondary Antibody-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100  $\mu$ L of **Substrate Solution** to each well. Incubate for 4 - 10 minutes on microplate shaker at room temperature. **Protect from light.**
11. The blue color in the wells should be determined the optical density of each well within 1 minute, using a microplate reader set to 650 nm.

## CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL STANDARD CURVE

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

STANDARD (NG/ML)	AVERAGE OD650 NM (CORRECTED)
Blank	0 (0.040)
1.95	0.032
7.8	0.105
31.25	0.379
125	1.015
500	2.302

## SPECIFICITY

Proteins	Cross-reactivity
Human Urine Uromodulin	100%
Human KIM-1	0
Human NGAL	0
Human Albumin	0
Human Hemopexin	0
Human TIMP-1	0
Human IGFBP-7	0
Human RBP4	0

Human uromodulin recombinant protein derived from E. Coli expression or Sf21 cells expression may NOT be detected by this kit.

SUMMARY OF ASSAY PROCEDURE

<b>PREPARE REAGENTS, SAMPLES AND STANDARDS</b>
↓
Add 100 µL of standard dilutions or samples to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Secondary Antibody-HRP Conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. <b>Protect from light.</b>
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 4-10 min on plate shaker at RT. <b>Protect from light.</b>
↓
Add 100 µL Stop Solution to each well. Read 650nm within 3 min.

