Version 26-05/24

User's Manual

Human Uromodulin ELISA Kit

REF **DEIA4842**

Σ **96T**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Human Uromodulin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human uromodulin.

General Description

Uromodulin (Tamm-Horsfall protein, UMOD) is an 85-kDa glycoprotein that is produced in the thick ascending limb of Henle´s loop and early distal convoluted tubules of the nephron. It consists of 616 amino acids including 48 cysteine residues which form the disulfide bridges responsible for its complex 3-D structure. It is a transmembrane protein, which is secreted into the urine through proteolytic cleavage of the glycosylphosphatidylinositol (GPI) anchor. It belongs to the GPI family. Healthy individuals excrete about 20-70 mg of uromodulin per day, making in the most abundant protein in the urine. Uromodulin modulates cell adhesion and signal transduction by interacting with cytokines and it inhibits the aggregation of calcium crystals. By reducing calcium oxalate precipitation, uromodulin plays a protective role with respect to renal stone formation as demonstrated by recent studies on THP-deficient mice prone to nephrolithiasis. THP acts as a host defense factor against urinary tract infections induced by uropathogens such as Esherichia coli, Staphylococcus saphrophyticus, Proteus mirabilis and Klebsiela pneumonie. Uromodulin binds to type 1 fimbriae of Escherichia coli and thereby blocks colonization of urothelial cells. Tamm-Horsfall protein interacts with other molecules and cells including IL-1, IL-2, TNF, IgG, neuthrophils, lymphocytes and monocytes. Binding of uromodulin to neutrophils induces synthesis of IL-8, provokes the respiratory burst and degranulation and stimulates chemotaxis and phagocytosis. Recently, genome-wide association studies identified uromodulin as a risk factor for chronic kidney disease and hypertension. Mutations in the Uromodulin gene are associated with three autosomal dominant tubulo-interstitial nephropathies such as familial juvenile hyperuricemic nephropathy (FJHN), medullary cystic kidney disease (MCKD2) and glomerulocystic kidney disease (GCKD). These disorders are characterized by juvenile onset of hyperuricemia, gout and progressive renal failure.

Principles of Testing

In the Human Uromodulin ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human uromodulin antibody. After a 60 minutes incubation followed by washing, biotin labelled polyclonal anti-human uromodulin antibody is added and incubated with the captured uromodulin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of uromodulin. A standard curve is constructed by plotting absorbance values against uromodulin concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Reagents And Materials Provided

Antibody Coated Microtiter Strips, ready to use, 96 wells

Biotin Labelled Antibody Conc. (10×), concentrated, 1.3 ml

Streptavidin-HRP Conjugate, ready to use, 13 ml

Master Standard, lyophilized, 2 vials

Quality Control HIGH, lyophilized, 2 vials

Quality Control LOW, lyophilized, 2 vials

Dilution Buffer, ready to use, 50 ml

Biotin-Ab Diluent, ready to use, 13 ml

Wash Solution Conc. (10×), concentrated, 100 ml

Substrate Solution, ready to use, 13 ml

Stop Solution, ready to use, 13 ml

Materials Required But Not Supplied

1. Deionized (distilled) water
2. Test tubes for diluting samples
3. Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
4. Precision pipettes to deliver 5-1000 µl with disposable tips
5. Multichannel pipette to deliver 100 µl with disposable tips
6. Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
7. Vortex mixer
8. Orbital microplate shaker capable of approximately 300 rpm
9. Microplate washer (optional). [Manual washing is possible but not preferable.]
10. Microplate reader with 450±10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
11. Software package facilitating data generation and analysis (optional)

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Specimen Collection And Preparation

The kit measures human uromodulin in serum, plasma (EDTA, citrate, heparin) and urine.

Samples should be assayed immediately after collection or should be stored at -20°C or -70°C. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze-thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

1. **Serum and plasma samples**

Dilute serum and plasma samples just prior to the assay 50x with Dilution Buffer, e.g. 5 µl of sample + 245 µl

of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

2. **Urine samples**

Dilute urine samples just prior to the assay 2 000x with Dilution Buffer in two steps as follows:

a. Dilution A (40×):

Add 5 µl of sample into 195 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

b. Dilution B (50×):

Add 5 µl of Dilution A into 245 µl of Dilution Buffer to prepare final dilution 2000×. **Mix well** (not to foam). Vortex is recommended.

Stability and storage: Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles. Urine samples should by stored at -70°C. **Do not store the diluted samples.**

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

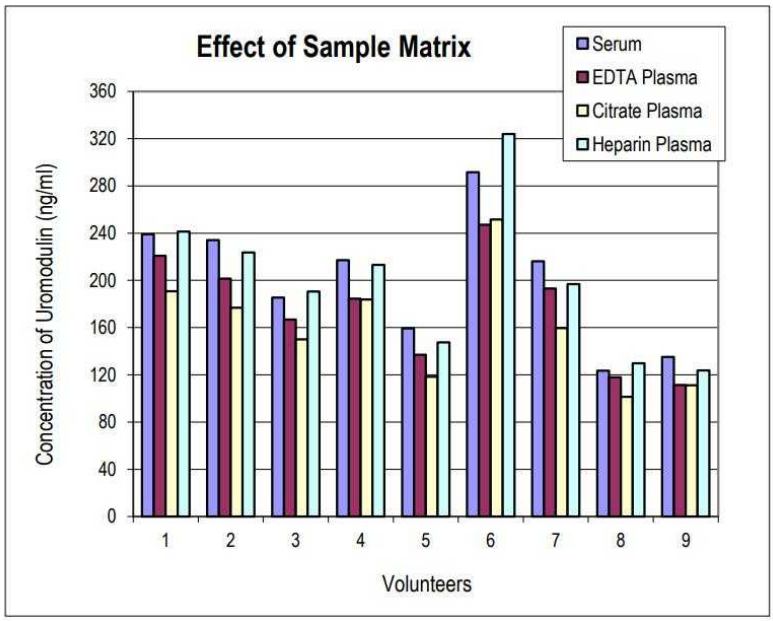
3. **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 9 individuals.

Results are shown below:

Uromodulin levels measured using Human Uromodulin ELISA in serum, EDTA, citrate and heparin plasma, respectively, from 9 individuals.

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	238.9	220.9	190.8	241.4
2	234.0	201.4	176.9	223.6
3	185.5	166.8	150.1	190.6
4	217.2	184.6	183.7	213.2
5	159.4	137.0	118.5	147.4
6	291.5	247.1	251.5	323.9
7	216.1	193.2	159.7	196.8
8	123.4	118.0	101.4	129.8
9	135.1	111.5	111.2	123.7
Mean (ng/ml)	**200**	**176**	**160**	**199**
Mean Plasma/Serum (%)		**88**	**80**	**99**
Coefficient of Determination R^2	**-**	**0.98**	**0.96**	**0.95**



4. **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C. However, no significant decline in concentration of human uromodulin was observed in serum samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.01%, respectively.

Sample	Incubation Temp, Period	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	-20°C	588.7	470.7	501.1	609.5
	2-8°C, 1 day	591.6	479.1	426.1	618.8
	2-8°C, 7 days	599.6	537.6	472.5	622.8
2	-20°C	281.6	221.9	207.1	267.9
	2-8°C, 1 day	271.0	247.9	211.7	276.0
	2-8°C, 7 days	269.0	248.3	174.5	271.0
3	-20°C	198.1	182.4	161.1	199.4
	2-8°C, 1 day	194.4	179.8	156.1	203.0
	2-8°C, 7 days	223.8	179.2	161.6	188.6

5. **Effect of Freezing/Thawing**

No significant decline was observed in concentration of human uromodulin in serum samples after repeated (5×) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	1x	175.3	168.2	139.2	167.1
	3x	167.8	169.4	146.6	176.7
	5x	175.3	155.6	144.8	150.8
2	1x	208.4	204.6	191.7	217.2
	3x	226.6	214.7	177.4	210.0
	5x	190.9	181.0	168.5	200.3
3	1x	145.8	135.2	155.9	130.0
	3x	139.0	146.6	163.4	134.6
	5x	148.8	152.9	159.4	133.6

Sample	Number of freezing / thawing cycles	Urine (ng/ml)
1	1x	18414
	3x	20086
	5x	17248
2	1x	9568
	3x	9186
	5x	8440
3	1x	18760
	3x	21576
	5x	18924

Plate Preparation

Example of a work sheet.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 32	QC HIGH	Sample 7	Sample 15	Sample 23	Sample 31
B	Standard 16	QC LOW	Sample 8	Sample 16	Sample 24	Sample 32
C	Standard 8	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Standard 4	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Standard 2	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Standard 1	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Standard 0.5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Reagent Preparation

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

1. **Assay reagents supplied ready to use:**

a. Antibody Coated Microtiter Strips

Stability and storage: Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

b. Streptavidin-HRP Conjugate, Dilution Buffer, Biotin-Ab Diluent, Substrate Solution, Stop Solution

Stability and storage: Opened reagents are stable 3 months when stored at 2-8°C.

2. **Assay reagents supplied concentrated or lyophilized:**

a. Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human uromodulin in the stock solution is **32 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	32 ng/ml
250 µl of stock	250 µl	16 ng/ml
250 µl of 16 ng/ml	250 µl	8 ng/ml
250 µl of 8 ng/ml	250 µl	4 ng/ml
250 µl of 4 ng/ml	250 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage: **Do not store the diluted Standard solutions.**

b. Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage: **Do not store the reconstituted Quality Controls.**

Note: Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control

that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

c. Biotin Labelled Antibody Conc. (10×)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (10×) with 9 parts Biotin-Ab Diluent. Example: 100 µl of Biotin Labelled Antibody Concentrate (10×) + 900 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage: Opened Biotin Labelled Antibody Concentrate (10×) is stable 3 months when stored at 2-8°C. **Do not store the diluted Biotin Labelled Antibody solution.**

d. Wash Solution Conc. (10×)

Dilute Wash Solution Concentrate (10×) ten-fold in distilled water to prepare a 1× working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage: The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10×) is stable 3 months when stored at 2-8°C.

Assay Procedure

1. Pipet **100 µl** of Standards, Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See Figure for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 min, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding 100 µl of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550-650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes following step 12.

    Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using

the values measured at 405 nm, is used to determine uromodulin concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

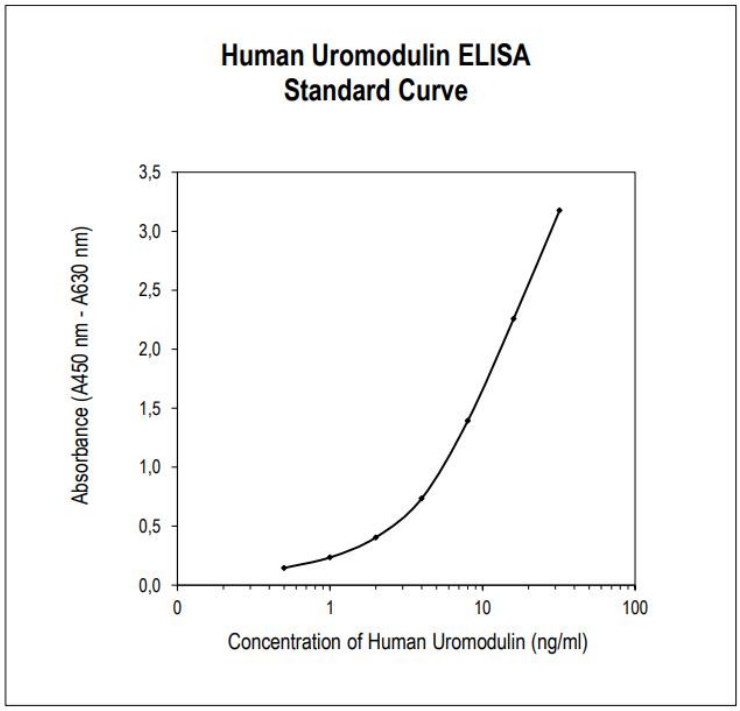
Calculation

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the absorbance (Y) of Standards against the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of uromodulin ng/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

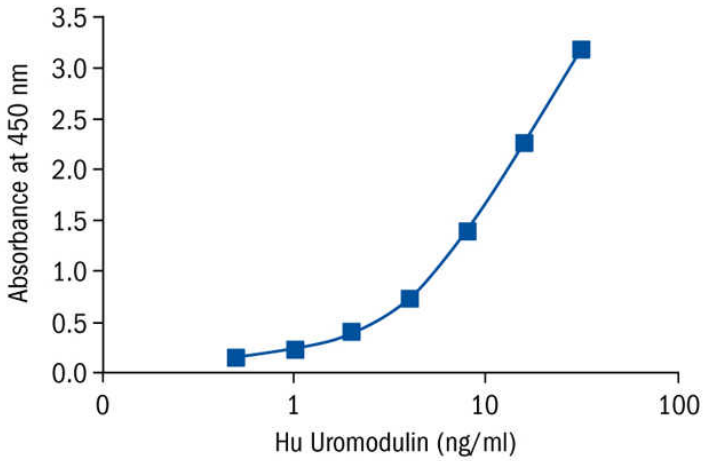
The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 2 ng/ml (from standard curve) × 50 (dilution factor) = 100 ng/ml.

Typical Standard Curve for Human Uromodulin ELISA.



Standard in this assay is human urine based.

Typical Standard Curve



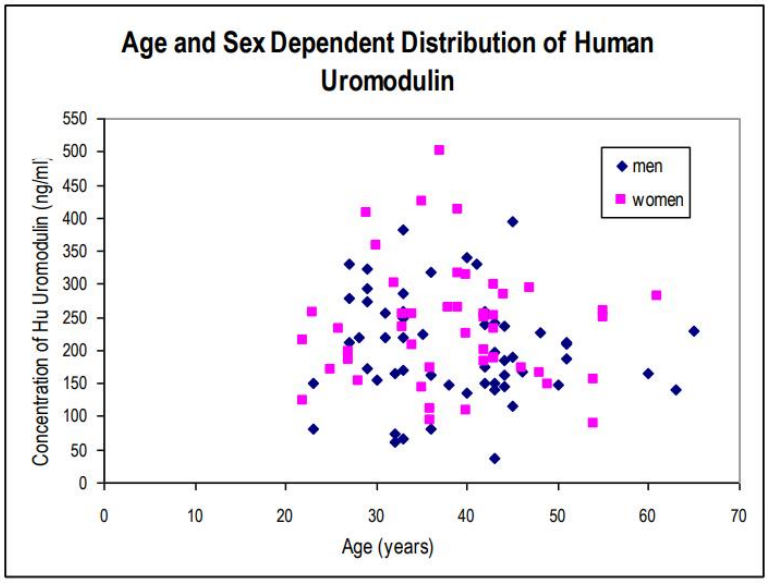
Reference Values

The following results were obtained when serum samples from 105 unselected donors (58 men + 47 women) 22-65 years old were assayed with the Biovendor Human Uromodulin ELISA in our laboratory:

1. **Age and Sex dependent distribution of uromodulin**

Human uromodulin concentration plotted against donor age and sex.

Sex	Age (years)	n	Mean	SD	Min.	Max.
			Uromodulin (ng/ml)			
Men	23-29	10	233.29	76.82	80.95	330.50
	30-39	19	197.48	86.56	62.65	382.65
	40-49	22	203.56	79.20	37.30	393.85
	50-65	7	184.34	31.92	139.50	229.75
Women	22-29	9	215.11	77.84	123.00	406.70
	30-39	14	269.14	110.61	92.90	501.15
	40-49	17	220.60	56.53	107.70	312.05
	50-61	5	206.24	73.34	88.20	282.25



2. **Uromodulin Levels in Nephropathy Patients**

Uromodulin levels were measured in serum and urine samples taken from 70 nephropathy patients (35 men, 35 women) and in 16 (serum) or 17 (urine) control samples using the Human Uromodulin ELISA. The following expected values were obtained (calculated as 5% and 95% percentile):

Serum:

Group	5%	95%
Controls	128,9	339,4
Patients	20,4	146,5

Urine:

Group	5%	95%
Controls	868	60028
Patients	556	13498

3. **Reference range**

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for uromodulin levels with the assay.

Performance Characteristics

For research use only!

The total assay time is less than 3.5 hours

The kit measures uromodulin in serum, plasma (EDTA, citrate, heparin) and urine

Assay format is 96 wells

Standard is native protein based

Quality Controls are human serum based

Components of the kit are provided ready to use, concentrated or lyophilized

Detection Range

0.5-32 ng/ml

Detection Limit

0.12 ng/ml

Sensitivity

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: Ablank + 3×SD_{blank}) is calculated from the real human uromodulin values in wells and is 0.09 ng/ml.

Dilution Buffer is pipetted into Blank wells.

Specificity

The antibodies used in this ELISA are specific for human uromodulin. We observed no interference of hemoglobin (2.0 mg/ml), bilirubin (0.2 mg/ml), triglycerides (10 mg/ml) and biotin (3500 ng/ml) on the measurement of uromodulin. Sera of several mammalian species were measured in the assay. See results below.

Note: The crossreactivity with dog urine was also observed.

Mammalian serum sample	Observed crossreactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	-	437.55	-	-
	2x	215.25	218.78	98.4
	4x	110.70	109.39	101.2
	8x	54.60	54.69	99.8
Serum 2	-	423.00	-	-
	2x	212.05	211.50	100.3
	4x	108.10	105.75	102.2
	8x	51.65	52.88	97.7
Urine 1	-	24186	-	-
	2x	11374	12093	94.1
	4x	6072	6047	100.4
	8x	3114	3023	103.0
Urine 2	-	14760	-	-
	2x	7388	7380	100.1
	4x	3680	3690	99.7
	8x	1842	1845	99.8
EDTA plasma	-	367.3	-	-
	2x	182.3	183.6	99.3
	4x	94.3	91.8	102.7
	8x	44.7	45.9	97.4
Citrate plasma	-	353.4	-	-
	2x	195.4	176.7	110.6
	4x	100.3	88.3	113.5
	8x	51.3	44.2	116.1
Heparin plasma	-	236.1	-	-
	2x	126.4	118.1	107.1
	4x	65.0	59.0	110.1
	8x	29.9	29.5	101.4

Recovery

Serum samples were spiked with different amounts of human uromodulin and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	94.5	-	-
	480.6	494.5	97.2
	283.0	294.5	96.1
	187.9	194.5	96.6
Serum 2	104.4	-	-
	506.4	504.4	100.4
	281.2	304.4	92.4
	186.6	204.4	91.3
Urine 1	6676	-	-
	21954	22676	96.8
	14744	14676	100.5
	10742	10676	100.6
Urine 2	4602	-	-
	20632	20602	100.1
	12210	12602	96.9
	8426	8602	98.0
EDTA plasma	193.1	-	-
	518.0	593.1	87.3
	345.6	393.1	87.9
	266.7	293.1	91.0
Citrate plasma	180.1	-	-
	485.4	581.1	83.7
	312.1	380.1	82.1
	231.0	280.1	82.5
Heparin plasma	85.7	-	-
	405.3	485.7	83.4
	236.2	285.7	82.7
	159.3	185.7	85.8

Reproducibility

1. Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	315.0	8.9	2.8
2	136.1	1.7	1.2

2. Inter-assay (Run-to-Run) (n=6)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	166.1	8.6	5.2
2	433.5	32.8	7.6

Precautions

1. **For professional use only**
2. Wear gloves and laboratory coats when handling immunodiagnostic materials
3. Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
4. This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. These materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
5. This kit contains components of animal origin. These materials should be handled as potentially infectious
6. Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
7. The materials must not be pipetted by mouth

Trouble Shooting

1. **Weak signal in all wells**

Possible explanations:

Omission of a reagent or a step

Improper preparation or storage of a reagent

Assay performed before reagents were allowed to come to room temperature

Improper wavelength when reading absorbance

2. **High signal and background in all wells**

Possible explanations:

Improper or inadequate washing

Overdeveloping: incubation time with Substrate Solution should be decreased before addition of Stop Solution

Incubation temperature over 30°C

3. **High coefficient of variation (CV)**

Possible explanation:

Improper or inadequate washing

Improper mixing Standards, Quality Controls or samples

Limitations

1. Reagents with different lot numbers should not be mixed
2. Use thoroughly clean glassware
3. Use deionized (distilled) water, stored in clean containers
4. Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for

each sample and reagent

5. Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
6. Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
7. Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements