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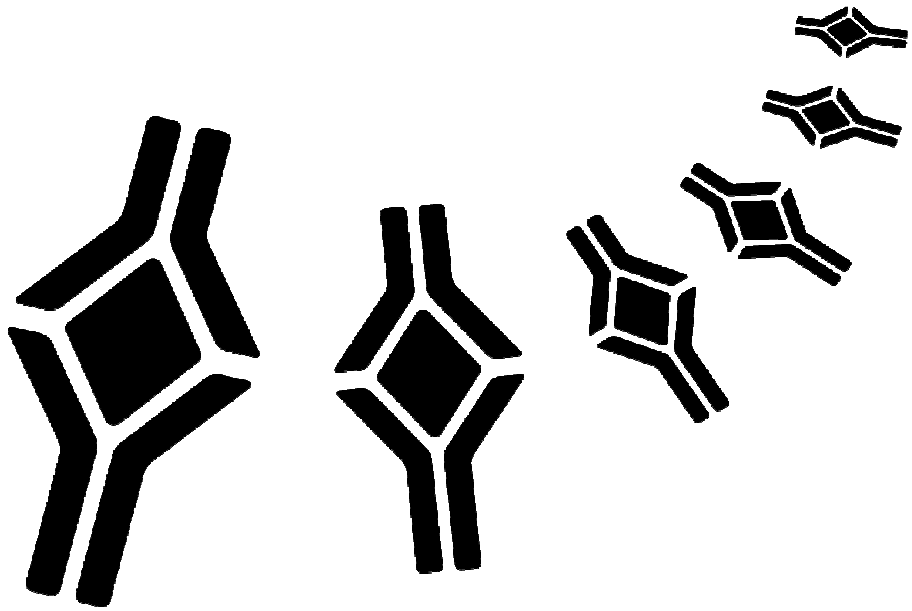

HUMAN UROMODULIN ELISA

Product Data Sheet

Cat. No.:RD191163200R

For Research Use Only

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» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191163200R Human Uromodulin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human uromodulin.

Features

- **It is intended 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

2. STORAGE, EXPIRATION

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

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

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.

<u>Areas of investigation:</u>
Urolithiasis
Urinary tract infections
Nephrophathies

4. TEST PRINCIPLE

In the Biovendor Human Uromodulin ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human uromodulin antibody. After a 60 minute 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.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- 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
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- 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
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- 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
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (10x)	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. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

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

9. PREPARATION OF REAGENTS

» **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**

- **Assay reagents supplied ready to use:**

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.

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.

- **Assay reagents supplied concentrated or lyophilized:**

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.

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.

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

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

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x 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 (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

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.

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.

Urine samples:
Dilute urine samples just prior to the assay 2 000x with Dilution Buffer in two steps as follows:
Dilution A (40x):
Add 5 µl of sample into 195 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (50x):
Add 5 µl of Dilution A into 245 µl of Dilution Buffer to prepare final dilution 2 000x. **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.

See Chapter 13 for stability of serum samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of human uromodulin.

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

11. 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 1* 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 2: 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.

	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

Figure 1: Example of a work sheet.

12. CALCULATIONS

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) x 50 (dilution factor) = 100 ng/ml.

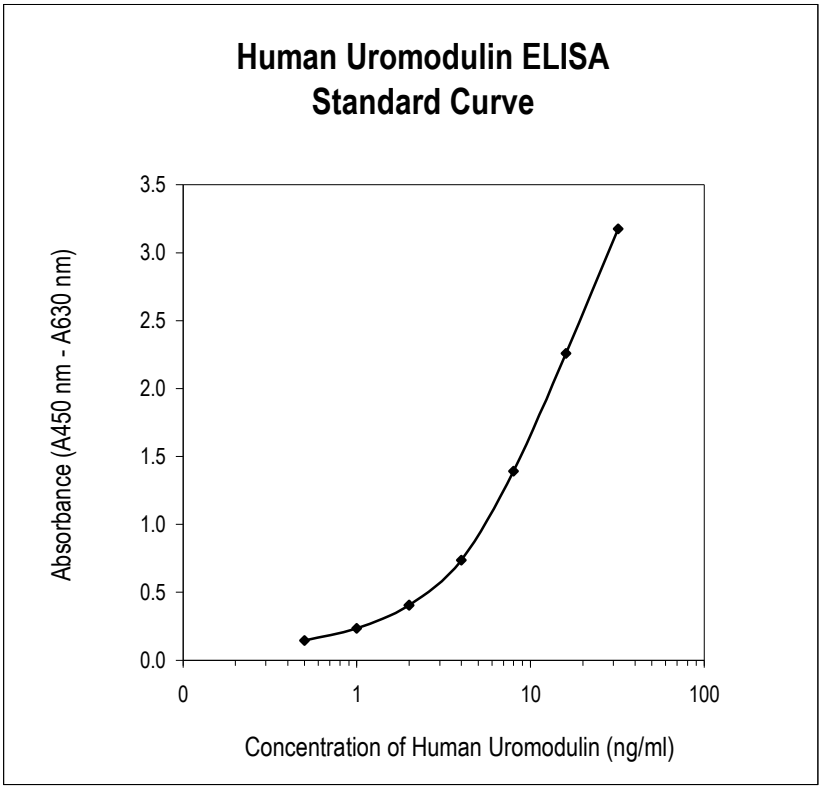

Figure 2: Typical Standard Curve for Human Uromodulin ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Uromodulin ELISA are presented in this chapter

- **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: A_{blank} + $3xSD_{blank}$) is calculated from the real human uromodulin values in wells and is 0.12 ng/ml.
* Dilution Buffer is pipetted into Blank wells.

- **Limit of Assay**

Results exceeding human uromodulin level of 32 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the uromodulin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human uromodulin.

Serum of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com

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

Note: The crossreactivity with dog urine was also observed.

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	314.98	8.93	2.8
2	136.08	1.68	1.2

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	166.07	8.59	5.2
2	433.47	32.76	7.6

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	94.45	-	-
	480.60	494.45	97.2
	282.95	294.45	96.1
	187.90	194.45	96.6
2	104.35	-	-
	506.40	504.35	100.4
	281.20	304.35	92.4
	186.55	204.35	91.3

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	437.55	-	-
	2x	215.25	218.78	98.4
	4x	110.70	109.39	101.2
	8x	54.60	54.69	99.8
2	-	423.00	-	-
	2x	212.05	211.50	100.3
	4x	108.10	105.75	102.2
	8x	51.65	52.88	97.7

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.
Results are shown below:

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	86.8	75.7	68.9	214.4
5	217.2	184.6	183.7	213.2
6	159.4	137.0	118.5	147.4
7	291.5	247.1	251.5	323.9
8	216.1	193.2	159.7	196.8
9	123.4	118.0	101.4	129.8
10	135.1	111.5	111.2	123.7
Mean (ng/ml)	**188.8**	**165.6**	**151.3**	**200.5**
Mean Plasma/Serum (%)		**87.7**	**80.1**	**106.2**
Coefficient of Determination R^2	**-**	**0.97**	**0.97**	**0.97**

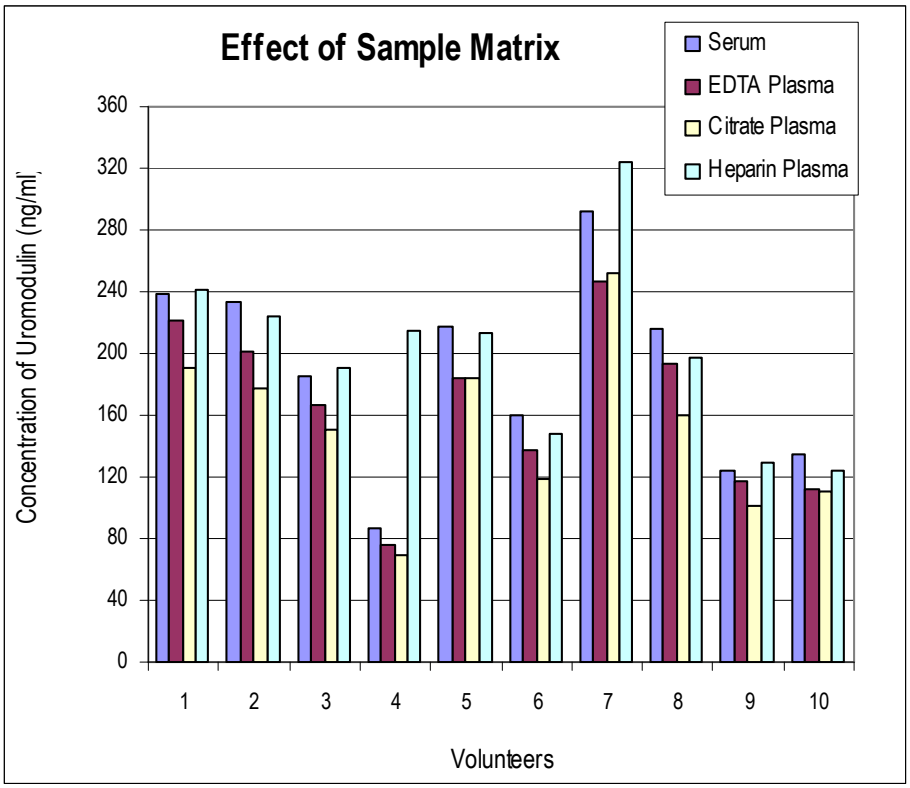

Figure 3: Uromodulin levels measured using Human Uromodulin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **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

- **Effect of Freezing/Thawing**

No significant decline was observed in concentration of human uromodulin in serum samples after repeated (5x) 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

14. DEFINITION OF THE STANDARD

Standard in this assay is human urine based.

15. PRELIMINARY POPULATION AND CLINICAL DATA

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:

- **Age and Sex dependent distribution of uromodulin**

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

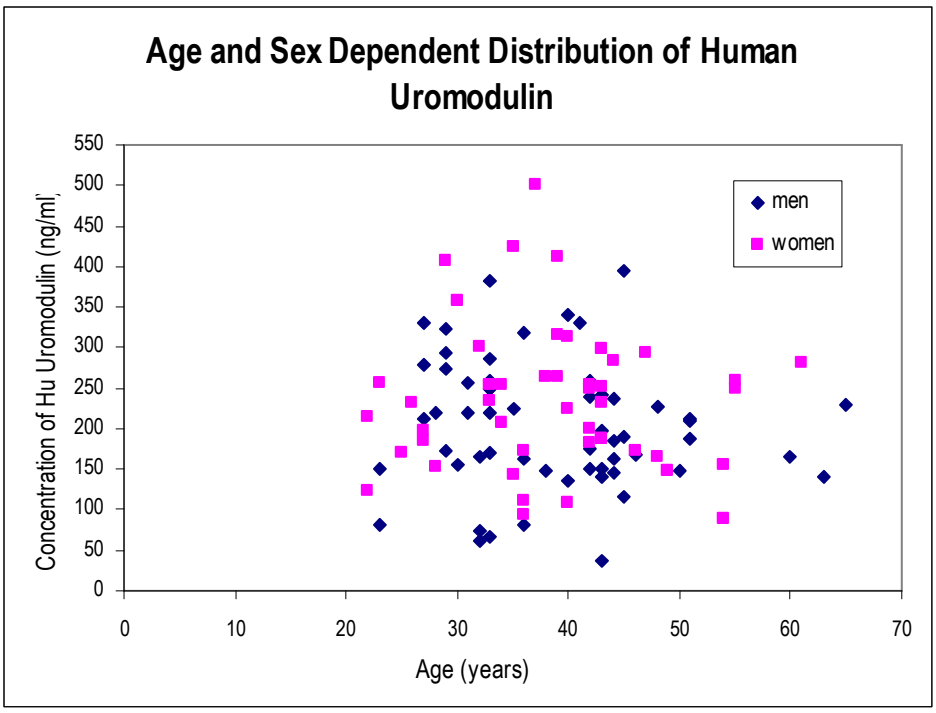

Figure 5: Human uromodulin concentration plotted against donor age and sex.

- **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.

16. TROUBLESHOOTING AND FAQS

›› 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

›› 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

›› High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

17. REFERENCES

References to human uromodulin:

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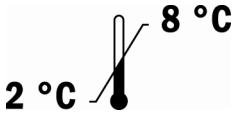
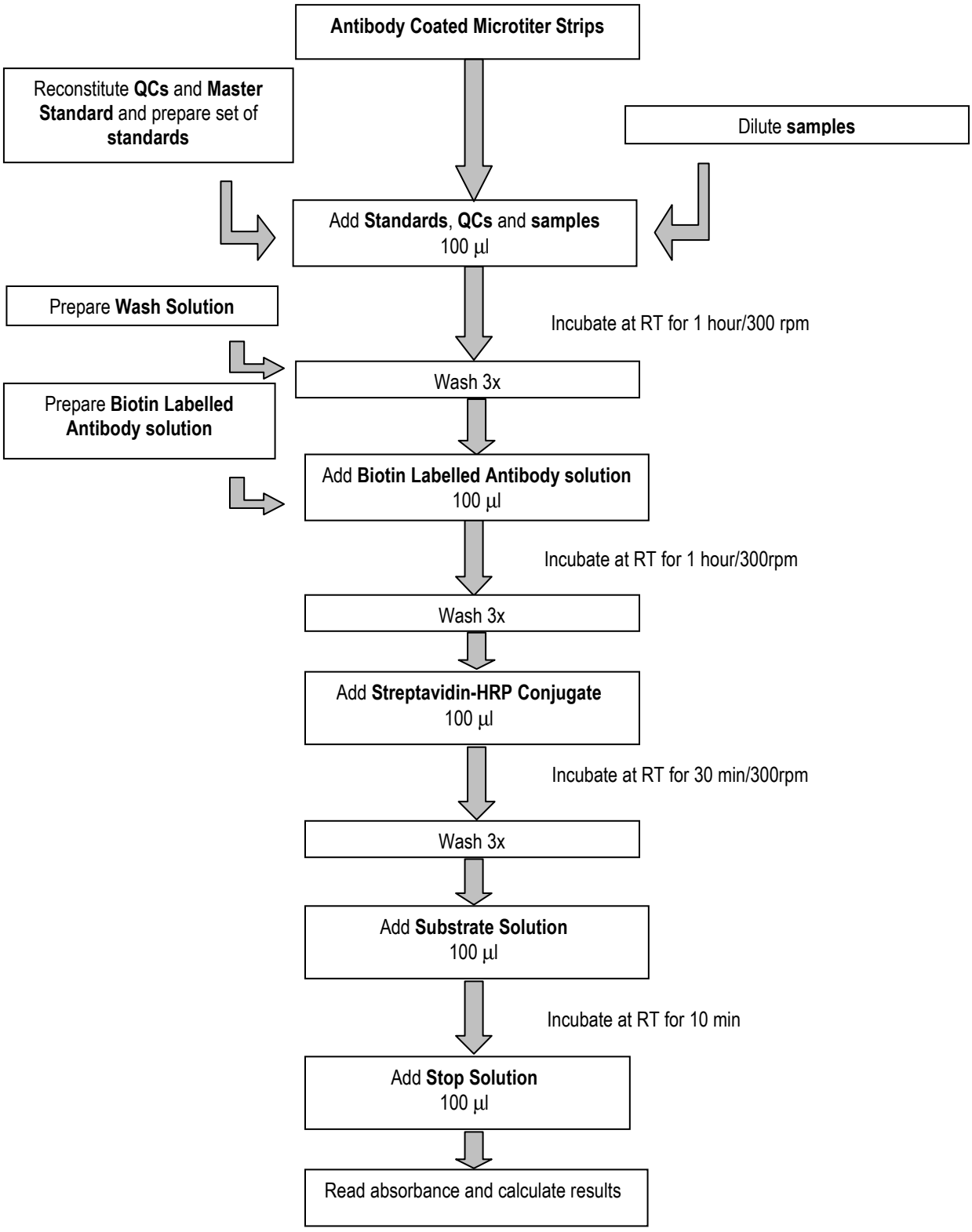
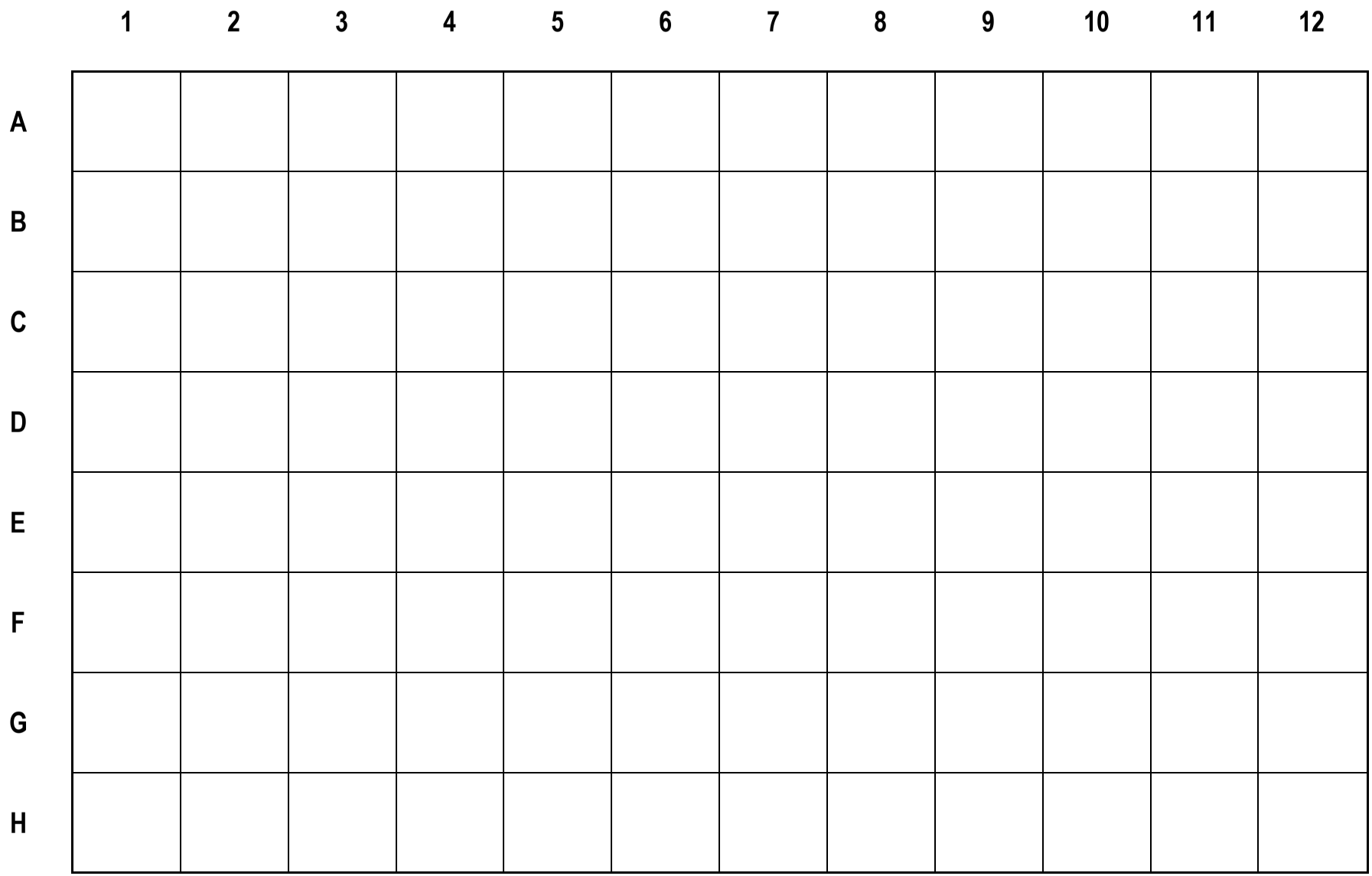
For more references on this product see our WebPages at www.biovendor.com

18. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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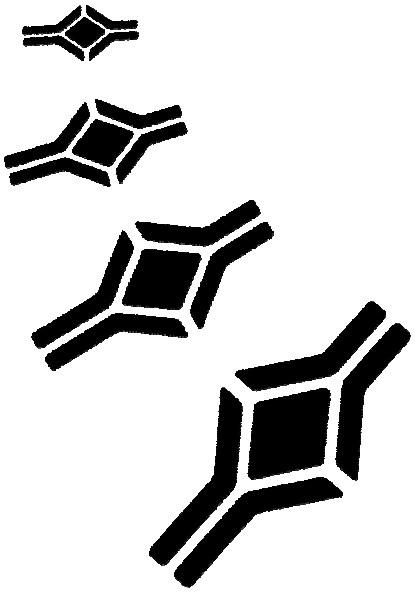

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