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Research
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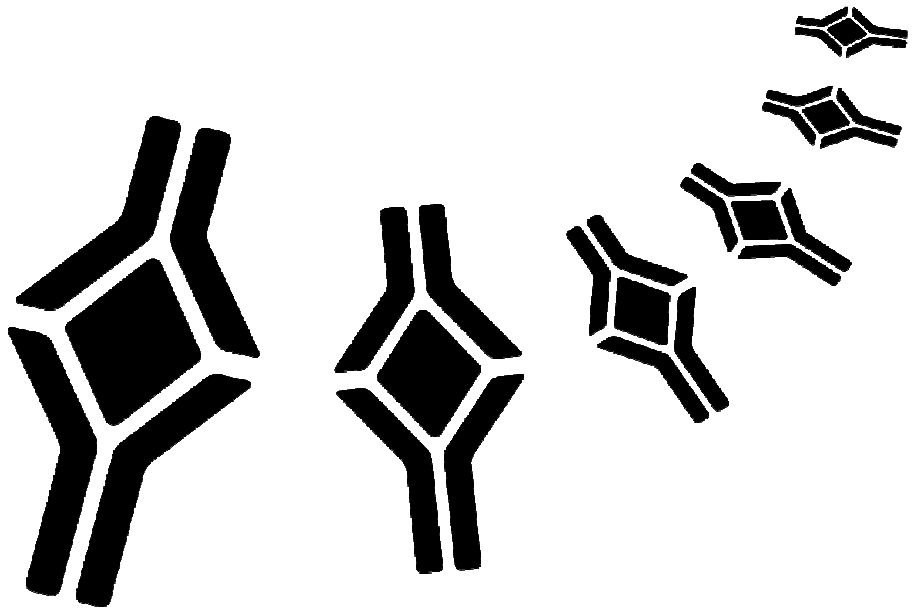

HUMAN PROUROGUANYLIN ELISA

Product Data Sheet

Cat. No.: RD191069200R

For Research Use Only

CONTENTS

» **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 RD191069200R Human Prouroguanylin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human prouroguanylin in serum and plasma.

Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures prouroguanylin in serum, plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein 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

Prouroguanylin (about 9.7 kDa) is a biologically inactive form of uroguanylin circulating in a bloodstream. Uroguanylin is a small-molecular-weight peptide which has been shown to participate in the regulation of salt and water homeostasis in mammals via cGMP-mediated processes in the intestine, kidney and other epithelia.
Prouroguanylin levels are markedly increased in chronic renal failure. The severity of chronic renal disease correlates with the magnitude of increases in plasma prouroguanylin concentrations.
Uroguanylin/prouroguanylin levels also increased in the nephrotic syndrome. It may be concluded that uroguanylin/prouroguanylin is cleared from the circulation by the kidney and that reduced functioning of renal mass and decreased glomerular filtration rates (GFR) lead to substantial increases in the concentrations of these peptides in serum and plasma. Circulating forms of uroguanylin and prouroguanylin are thought to be a major source of the urinary forms of biologically active uroguanylin. Both of these peptides can enter renal tubules by glomerular filtration. Prouroguanylin in the tubular lumen is then converted to active uroguanylin by tubular endoproteases because prouroguanylin is not detected in the urine.
Studies of pathogenesis of colorectal cancer demonstrate that prouroguanylin may serve as a marker of colon tumors in the body. Recent experiments also refer to possibility of prouroguanylin to play a significant role in diagnostics and treatment of heart diseases.

Areas of investigation:
Renal disease
Heart failure
Oncology

4. TEST PRINCIPLE

In the BioVendor Human Prouroguanylin ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human prouroguanylin antibody. After 60 minutes incubation and washing, biotin-labelled polyclonal anti-human prouroguanylin antibody is added and incubated with captured prouroguanylin 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 prouroguanylin. A standard curve is constructed by plotting absorbance values against concentrations of prouroguanylin 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 may contain components of human or 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	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 ml
Wash Solution Concentrate (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 stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate
Biotin-Ab Diluent
Dilution Buffer
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:**

Human Prouroguanylin Master Standard:
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Master 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 prouroguanylin in the stock solution is **22 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	22 ng/ml
200 µl of stock	800 µl	4400 pg/ml
500 µl of std. 4400 pg/ml	500 µl	2200 pg/ml
500 µl of std. 2200 pg/ml	500 µl	1100 pg/ml
500 µl of std. 1100 pg/ml	500 µl	550 pg/ml
500 µl of std. 550 pg/ml	500 µl	275 pg/ml
500 µl of std. 275 pg/ml	500 µl	138 pg/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
The reconstituted Master Standard must be used immediately.
Do not store the diluted Standard solutions.

Biotin Labelled Antibody:
Refer to the Certificate of Analysis for current volume of distilled water needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute reconstituted Biotin Labelled Antibody Concentrate (100x) with Biotin-Ab Diluent e.g. 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Concentrate (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 prouroguanylin in serum or plasma.

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.

Dilute samples 3x with Dilution Buffer just prior to the assay (e.g. 50 µl of sample + 100 µl of Dilution Buffer when assaying samples as singlets or preferably 100 µl of sample + 200 µl of Dilution Buffer for duplicates). **Mix well** (not to foam). Vortex is recommended.

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

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of Prouroguanylin.

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 prepared Standards, 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 **5-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 Labeled Antibody 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 **5-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 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells **5-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 **15 minutes** at room temperature. The incubation time may be extended [up to 30 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.**

<u>Note 1:</u> If some samples and standard/s have absorbance 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 prouroguanylin 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.

<u>Note 2:</u> Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. 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 4400**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 2200**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 1100**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 550**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 275**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 138**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

Figure 1: Example of a work sheet.

12. CALCULATIONS

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

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of 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. 250 pg/ml (from standard curve) x 3 (dilution factor) = 750 pg/ml.

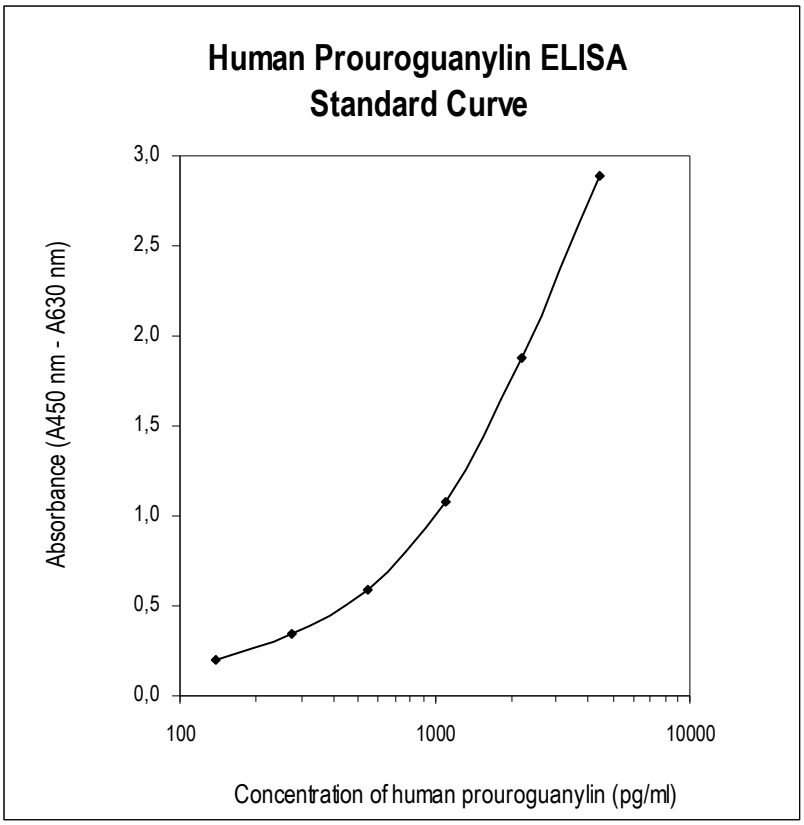

Figure 2: Typical Standard Curve for Human Prouroguanylin ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Prouroguanylin 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 prouroguanylin values in wells and is 47 pg/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

The antibodies used in this ELISA are specific for human prouroguanylin with no detectable crossreactivities to human proguanylin.

Sera of mammalian species were not tested in the assay.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	1449	18.0	1.3
2	911	29.0	3.2

Inter assay (Run-to-Run) (n=8)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	1583	88.0	5.6
2	3070	218.0	7.1

- **Spiking Recovery**

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

Sample	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
1	662	-	-
	991	996	99.6
	1275	1329	96.0
	1914	1996	95.9
2	509	-	-
	870	842	103.4
	1168	1175	99.3
	1745	1842	94.7

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
1	-	3417	-	-
	2x	1835	1709	107.4
	4x	1016	854	118.9
	8x	474	427	110.9
2	-	2982	-	-
	2x	1558	1491	104.5
	4x	802	745	107.6
	8x	391	373	104.8

- **Effect of sample matrix**

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

Volunteer No.	*Serum (pg/ml)*	*Plasma (pg/ml)*		
		EDTA	*Citrate*	*Heparin*
1	1229	1053	1209	1129
2	1158	1098	984	1281
3	1269	1204	1395	1270
4	3463	2841	2460	2968
5	1329	1354	1015	1251
6	1169	1176	1024	1131
7	906	939	723	891
8	688	856	600	744
9	645	643	486	596
10	1079	993	854	973
Mean (ng/ml)	**1293**	**1216**	**1075**	**1223**
Mean Plasma/Serum (%)	**-**	**94.0**	**83.1**	**94.6**
Correlation coef. R^2	**-**	**0.99**	**0.93**	**0.99**

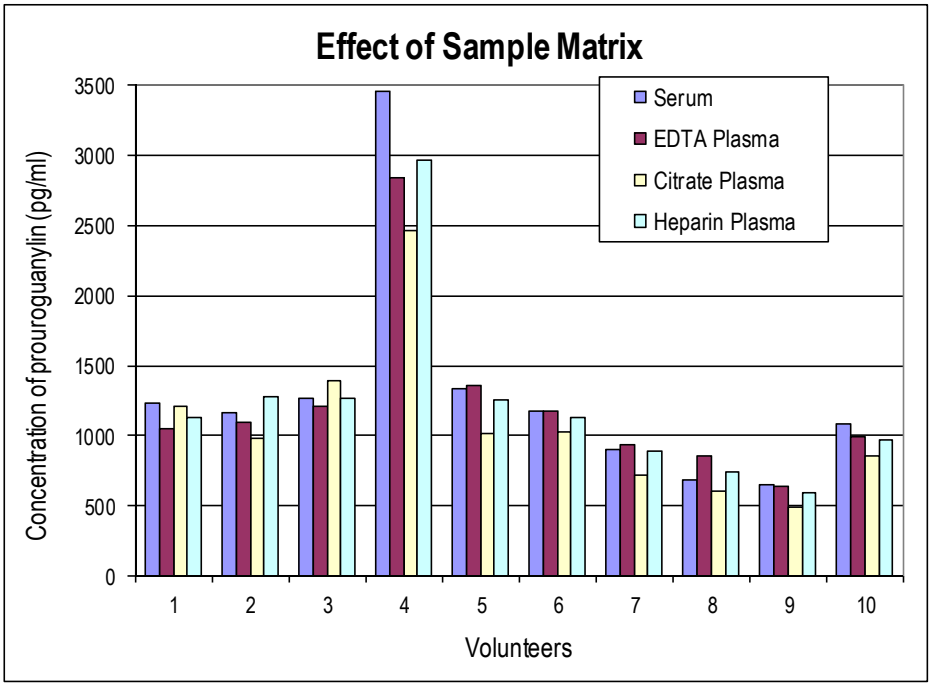

Fig. 3: Prouroguanylin levels measured using Human Prouroguanylin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

The recombinant human protein prouroguanylin (10.7 kDa), produced in *E. coli*, is used as the Standard.

Starting from lot E15-117 (November 2015), the method of determination of protein concentration in the Standard has been changed. The decline in resulting protein concentration is reflected by the shift of the standard curve from 0.8 – 40 ng/ml to 138 – 4 400 pg/ml which also affects the final calculated concentration values for samples. Thus, to compare the results obtained with the previous version (up to the lot E15-075) to the results measured with the current version, the former have to be **divided by 9** and then converted into **pg/ml by multiplying by 1000.**

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 152 unselected donors (65 women + 87 men), 21-65 years old were assayed with Biovendor Human Prouroguanylin ELISA kit in our laboratory.

- **Age and Sex - Dependent Distribution of Prouroguanylin Values**

Sex	*Age (years)*	*n*	*prouroguanylin (pg/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	21-29	16	1595	1580	422	708	2760
	30-39	25	1426	1468	503	465	2682
	40-49	30	1380	1233	414	730	2356
	50-65	20	3464	1556	244	1040	1939
Women	22-29	12	1084	1046	375	563	1850
	30-39	25	1316	1212	499	705	3314
	40-49	20	1311	1276	496	588	2533
	50-61	8	1505	1568	194	1250	1815

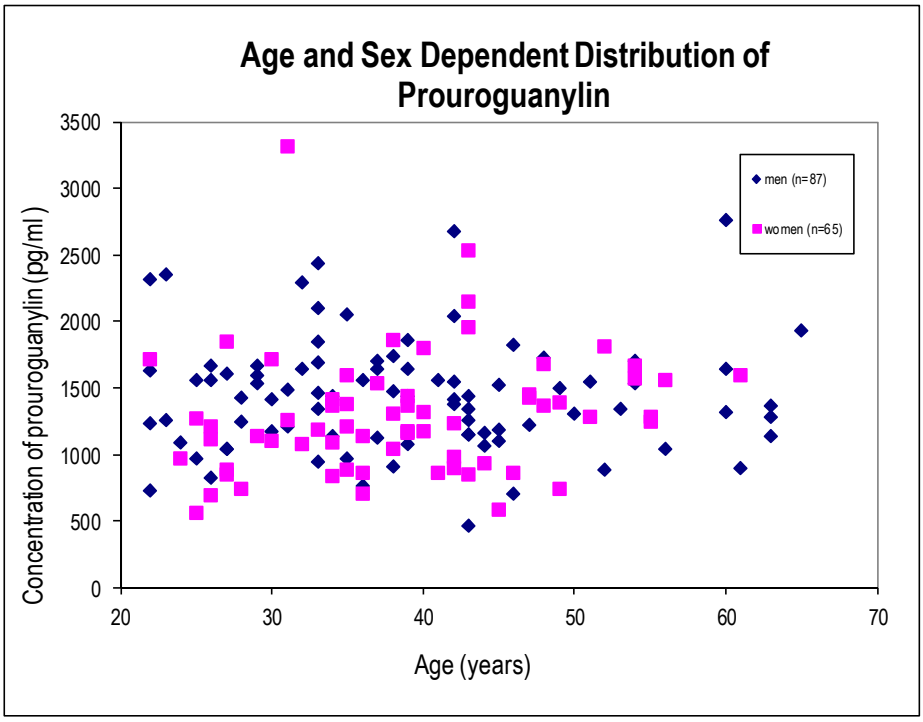

Fig. 4: Human prouroguanylin concentration plotted against donor age and sex

- **Reference range**

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 prouroguanylin levels with the assay.

16. METHOD COMPARISON

BioVendor Human Prouroguanylin ELISA has not been compared to any other immunoassay.

17. 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 or samples

18. REFERENCES

References to Human Prouroguanylin ELISA:

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- Forte LR: A novel role for uroguanylin in the regulation of sodium balance. *Am Clin Invest* **112**, 1138-1141 (2003).
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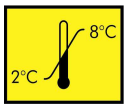
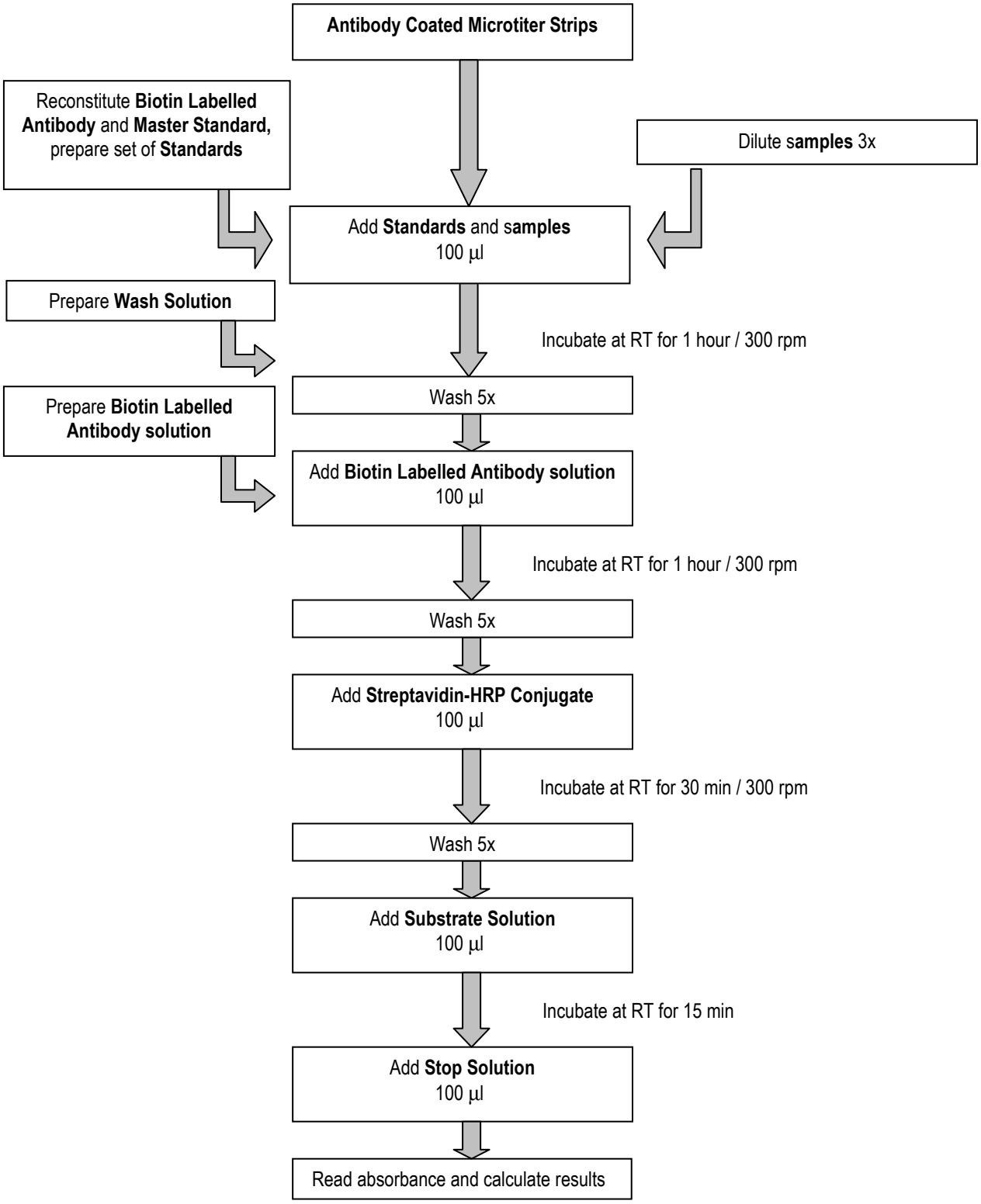
- Shailubhai K, Yu HH, Karunanandaa K, Wang JY, Eber SL, Wang Y, Joo NS, Kim HD, Miedema BW, Abbas SZ, Boddupalli SS, Currie MG, Forte LR: Uroguanylin Treatment Suppresses Polyp Formation in the *APC$^{Min/+}$* Mouse and Induces Apoptosis in Human Colon Adenocarcinom Cells via Cyclic GMP. *Cancer research* **60**, 5151-5157 (2000).

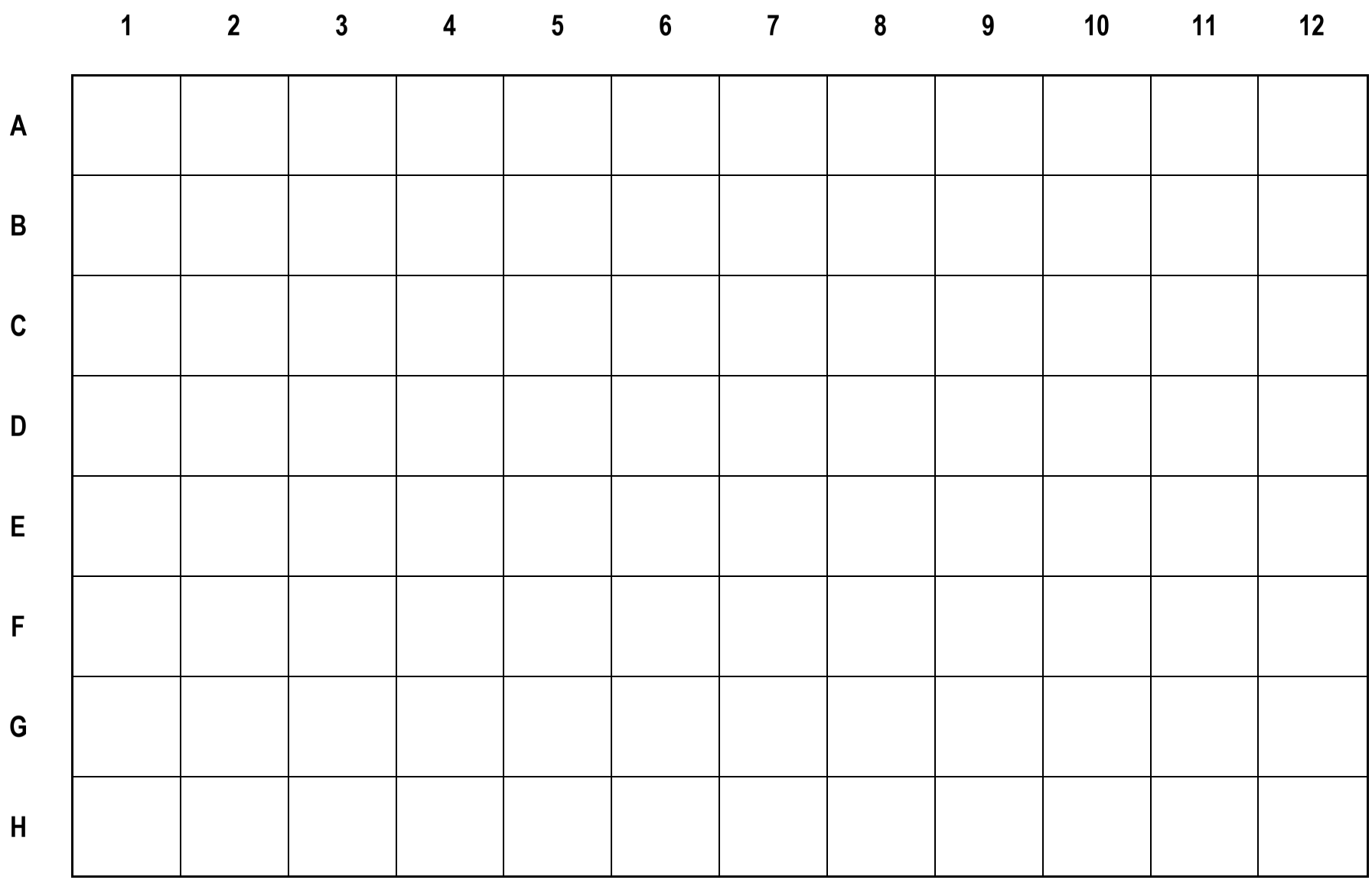
For more references on this product see our WebPages at www.biovendor.com

19. 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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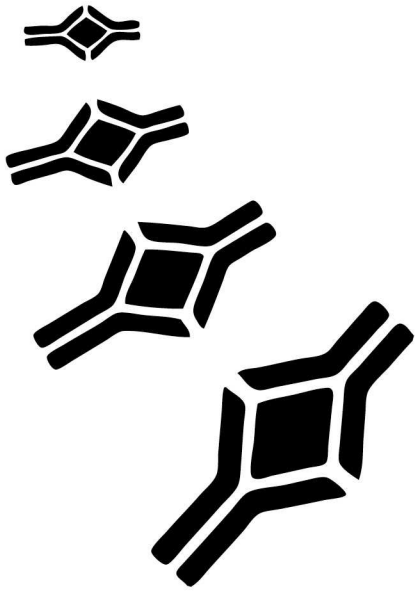

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