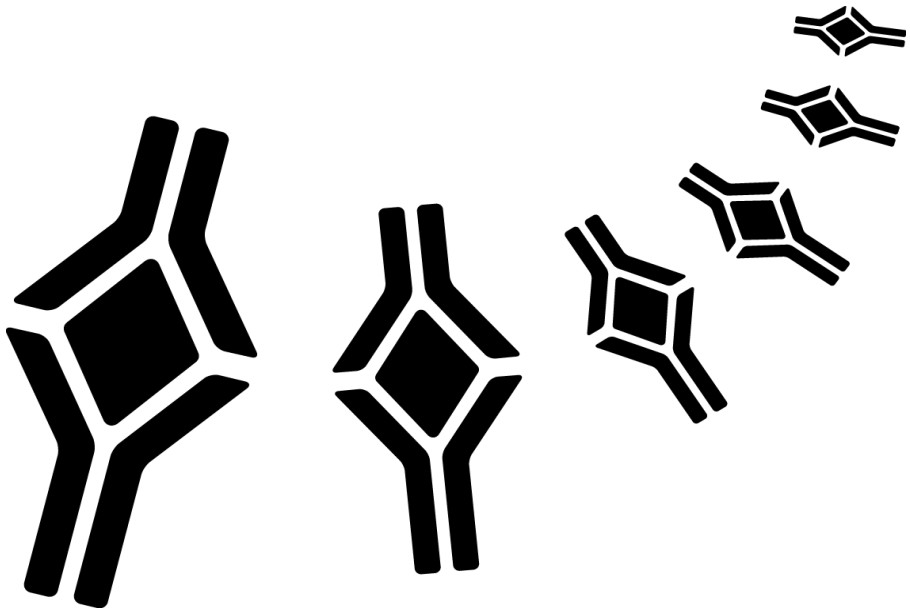
BioVendor
Research
and Diagnostic Products



HUMAN PROSTAGLANDIN D SYNTHASE (LIPOCALIN-TYPE) ELISA

Product Data Sheet

Cat. No.: RD191113100R

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 RD191113100R Human Prostaglandin D Synthase (Lipocalin-type) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human prostaglandin D synthase (L-PGDS, beta-trace protein, BTP).

» Features

- **For research use only**
- The total assay time is less than 3 hours
- The kit measures prostaglandin D synthase in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid (CSF)
- Assay format is 96 wells
- Quality Controls are human serum based
- 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

Lipocalin–type prostaglandin D synthase (beta–trace protein) catalyzes the conversion of PGH2 to PGD2, a prostaglandin involved in smooth muscle contraction/relaxation and a potent inhibitor of platelet aggregation, involved in a variety of CNS functions, such as sedation, NREM sleep regulation, and allergic and inflammatory responses.
The serum and urinary levels have been proposed as an alternative marker for GFR and renal disease in the patients with diabetes, various renal diseases and renal transplant patients. In contrast to cystatin C, the levels of L-PGDS might not be influenced by the corticosteroid treatment, age and gender.
The elevated levels of L-PGDS in serum might be highly associated with hypertension and increasing numbers of the traditional atherosclerotic risk factors (e.g. C-IMT_{max}, HDL). It was shown in vitro that L-PGDS enhances insulin stimulated GLUT-4 translocation and probably is more effective than insulin alone at recruiting GLUT-4 to the plasma membrane.

Areas of investigation:
Renal disease
Heart disease
Neurological disease

4. TEST PRINCIPLE

In the BioVendor Human Prostaglandin D Synthase (Lipocalin-type) ELISA, standards, quality controls and samples are incubated in microtitrate plate wells pre-coated with polyclonal anti-human L-PGDS antibody. After 60 minutes incubation and washing, polyclonal anti-human L-PGDS antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated with captured L-PGDS for 60 minutes. Following another washing step, the remaining HRP 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 L-PGDS. A standard curve is constructed by plotting absorbance values against concentrations of L-PGDS 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. However, 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
Conjugate Solution	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	1 vial
Quality Control LOW	lyophilized	1 vial
Dilution Buffer	ready to use	2 x 20 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 10-1000 µl with disposable tips
- Multichannel pipette to deliver 50-100 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.

Conjugate Solution
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 Prostaglandin D Synthase 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 prostaglandin D synthase in the stock solution is **40 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	40 ng/ml
250 µl of std. 40 ng/ml	250 µl	20 ng/ml
250 µl of std. 20 ng/ml	250 µl	10 ng/ml
250 µl of std. 10 ng/ml	250 µl	5 ng/ml
200 µl of std. 5 ng/ml	300 µl	2 ng/ml
250 µl of std. 2 ng/ml	250 µl	1 ng/ml

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

Stability and storage:

Standard stock solution (40 ng/ml) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

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).

Dilute Quality Controls prior to use 100x with Dilution Bufffer in two steps as follows:

Dilution A (10x):

Add 10 µl of Quality Control into 90 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.

Dilution B (10x):

Add 15 µl of Dilution A into 135 µl of Dilution Buffer to prepare final dilution 100x and **mix well** (not to foam). Vortex is recommended. **For singlets.**

or

Dilution A (10x):

Add 10 µl of Quality Control into 90 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.

Dilution B (10x):

Add 25 µl of Dilution A into 225 µl of Dilution Buffer to prepare final dilution 100x and **mix well** (not to foam). Vortex is recommended. **For duplicates.**

Stability and storage:

Reconstitued Quality Controls should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted 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.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in 900 ml of distilled water to prepare a 1x working solution, e.g. 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 prostaglandin D synthase in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid (CSF).

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

Dilution of serum, plasma and urine:

Dilute samples prior to use 100x with Dilution Bufffer in two steps as follows:

Dilution A (10x):

Add 10 µl of sample into 90 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.

Dilution B (10x):

Add 15 µl of Dilution A into 135 µl of Dilution Buffer to prepare final dilution 100x and **mix well** (not to foam). Vortex is recommended. **For singlets.**

or

Dilution A (10x):

Add 10 µl of sample into 90 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.

Dilution B (10x):

Add 25 µl of Dilution A into 225 µl of Dilution Buffer to prepare final dilution 100x and **mix well** (not to foam). Vortex is recommended. **For duplicates.**

Dilution of CSF:

Dilute samples prior to use 500x with Dilution Bufffer in two steps as follows:

Dilution A (10x):

Add 10 µl of sample into 90 µl of Dilution Buffer and **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 500x and **mix well** (not to foam). Vortex is recommended. **For both singlets and duplicates.**

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 stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of L-PGDS.

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 diluted Standards, Quality Controls, Dilution Buffer (=Blank) and 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 Conjugate 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 Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. 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 with the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. 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 9.**

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 L-PGDS concentration of offscale 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 40**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 20**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 10**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 2**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 1**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

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 L-PGDS 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 Quality Controls and samples calculated from the standard curve must be multiplied by their respective dilution factor, because Quality Controls and samples have been diluted prior to the assay, e.g. 9.25 ng/ml (from standard curve) x 100 (dilution factor for serum) = 925 ng/ml = 0.925 μg/ml.

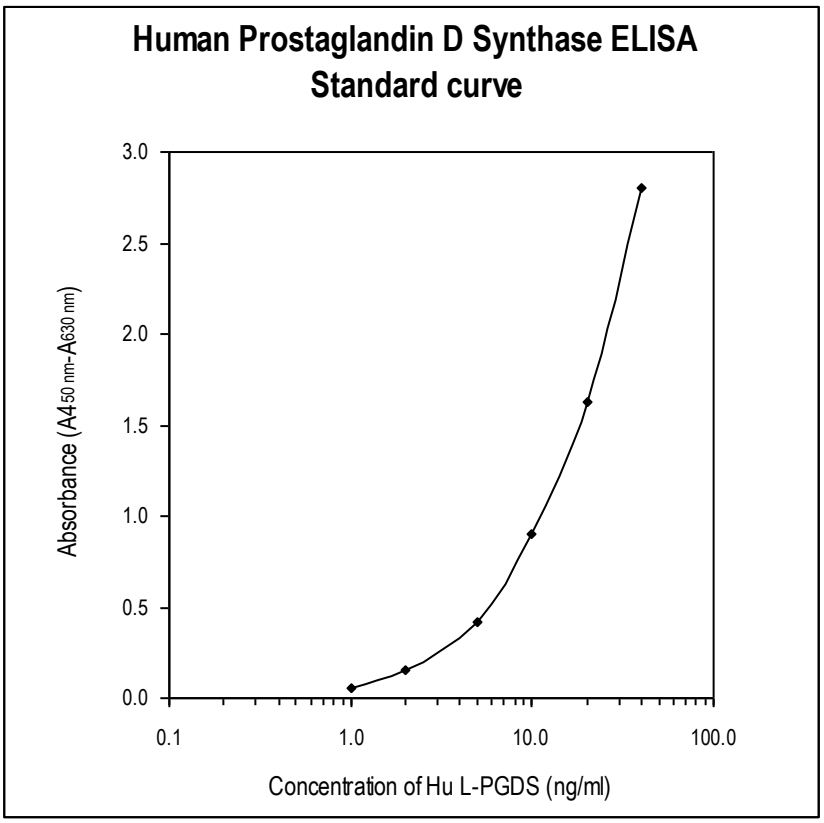


Figure 2: Typical Standard Curve for Human Prostaglandin D Synthase (Lipocalin-type) ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Prostaglandin D Synthase (Lipocalin-type) 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 prostaglanin D synthase values in wells and is 0.5 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding L-PGDS level of 40 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the L-PGDS concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human lipocalin-type prostaglandin D synthase.

Sera 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

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	343	0.13	3.73
2	2 660	1.18	4.42

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	314.4	0.131	3.83
2	2 225.2	1.011	4.54

- **Spiking Recovery**

Serum samples were spiked with different amounts of human prostaglandin D synthase, diluted with Dilution Buffer 100x and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	298	-	-
	487	498	97.7
	703	798	88.1
	1 169	1 298	90.1
2	522	-	-
	746	722	103.3
	834	1 022	81.5
	1 394	1 522	91.6

- **Linearity**

Serum samples were serially diluted with Dilution Buffer after primary dilution 100x and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	670	-	-
	2x	299	335	89.3
	4x	140	167	83.9
	8x	72	84	85.9
2	-	2 321	-	-
	2x	1 103	1 161	95.0
	4x	571	580	98.4
	8x	308	290	106.2

- **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	374.2	374.2	294.9	415.3
2	294.9	299.3	232.5	342.4
3	419.0	371.2	312.8	402.8
4	364.6	356.5	282.9	400.6
5	513.2	477.5	402.9	531.5
6	243.9	258.2	196.8	264.2
7	353.5	328.3	282.2	372.7
8	648.9	625.1	528.4	678.0
9	390.9	394.6	322.6	393.4
10	463.8	409.4	363.2	494.2
Mean (ng/ml)	**406.7**	**389.43**	**321.92**	**429.51**
Mean Plasma/Serum (%)	**-**	**96**	**83**	**133**
Coefficient of determination R^2	**-**	**0.98**	**0.99**	**0.99**

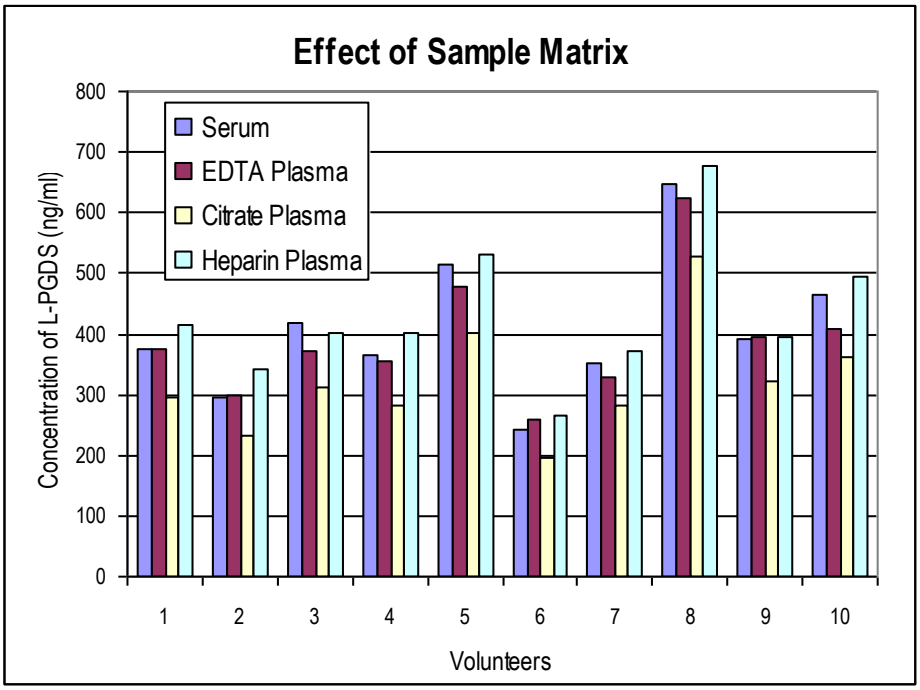


Figure 3: Prostaglandin D synthase levels measured using Human Prostaglandin D Synthase (Lipocalin-type) ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at -20°C. However, no decline in concentration of L-PGDS was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	*Incubation Temp, Period*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	427.1	403.0	339.3	488.8
	2-8°C, 1 day	441.3	417.9	345.0	473.2
	2-8°C, 7 days	442.7	405.9	340.7	435.6
2	-20°C	324.4	264.8	216.4	291.8
	2-8°C, 1 day	298.2	281.2	261.3	309.5
	2-8°C, 7 days	306.7	287.5	224.3	302.4
3	-20°C	286.3	254.7	183.7	309.5
	2-8°C, 1 day	317.7	256.1	209.6	238.8
	2-8°C, 7 days	272.0	235.8	199.9	275.1

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human prostaglandin D synthase in serum and plasma 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	250.4	278.4	217.5	278.9
	3x	272.1	268.8	216.0	279.4
	5x	276.0	259.6	221.4	276.5
2	1x	281.1	316.3	247.9	290.0
	3x	287.5	300.1	247.5	303.3
	5x	306.6	302.9	247.9	296.5
3	1x	250.4	278.9	217.5	278.4
	3x	272.1	268.8	216.0	279.4
	5x	276.0	259.6	221.4	276.5

- **Reference range**

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for L-PGDS levels with the assay.

14. DEFINITION OF THE STANDARD

A recombinant protein is used as the Standard. The recombinant human prostaglandin D synthase (lipocalin-type) is a 20.3 kDa protein containing 182 amino acid residues.

15. METHOD COMPARISON

The BioVendor Human Prostaglandin D Synthase (Lipocalin-type) ELISA was compared to the commercial latex nephelometric assay, by measuring 70 serum and urine samples. The following correlation graphs were obtained.

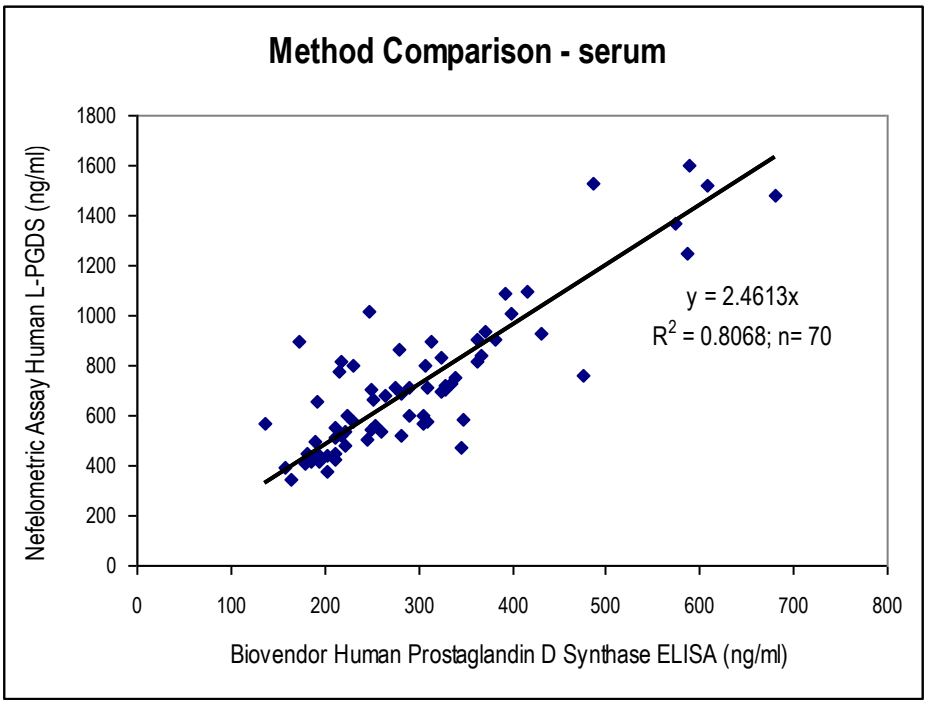


Figure 4: Method comparison in serum.

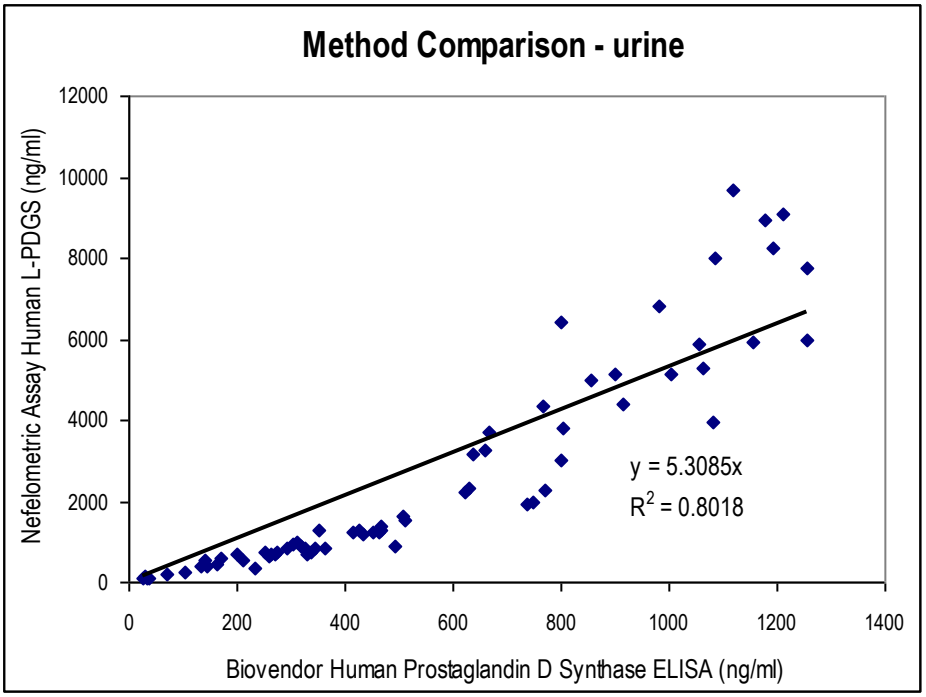


Figure 5: Method comparison in urine.

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

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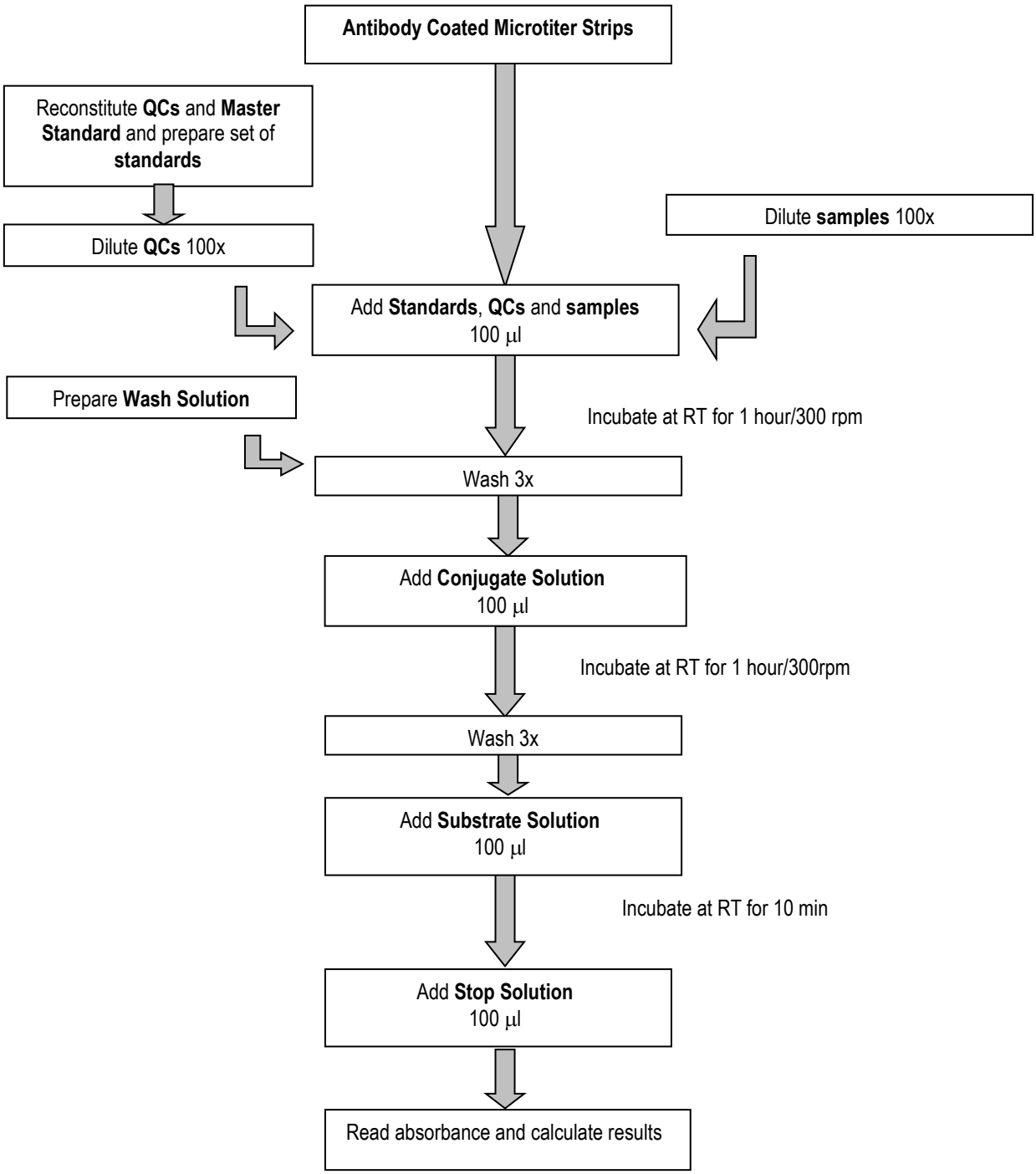
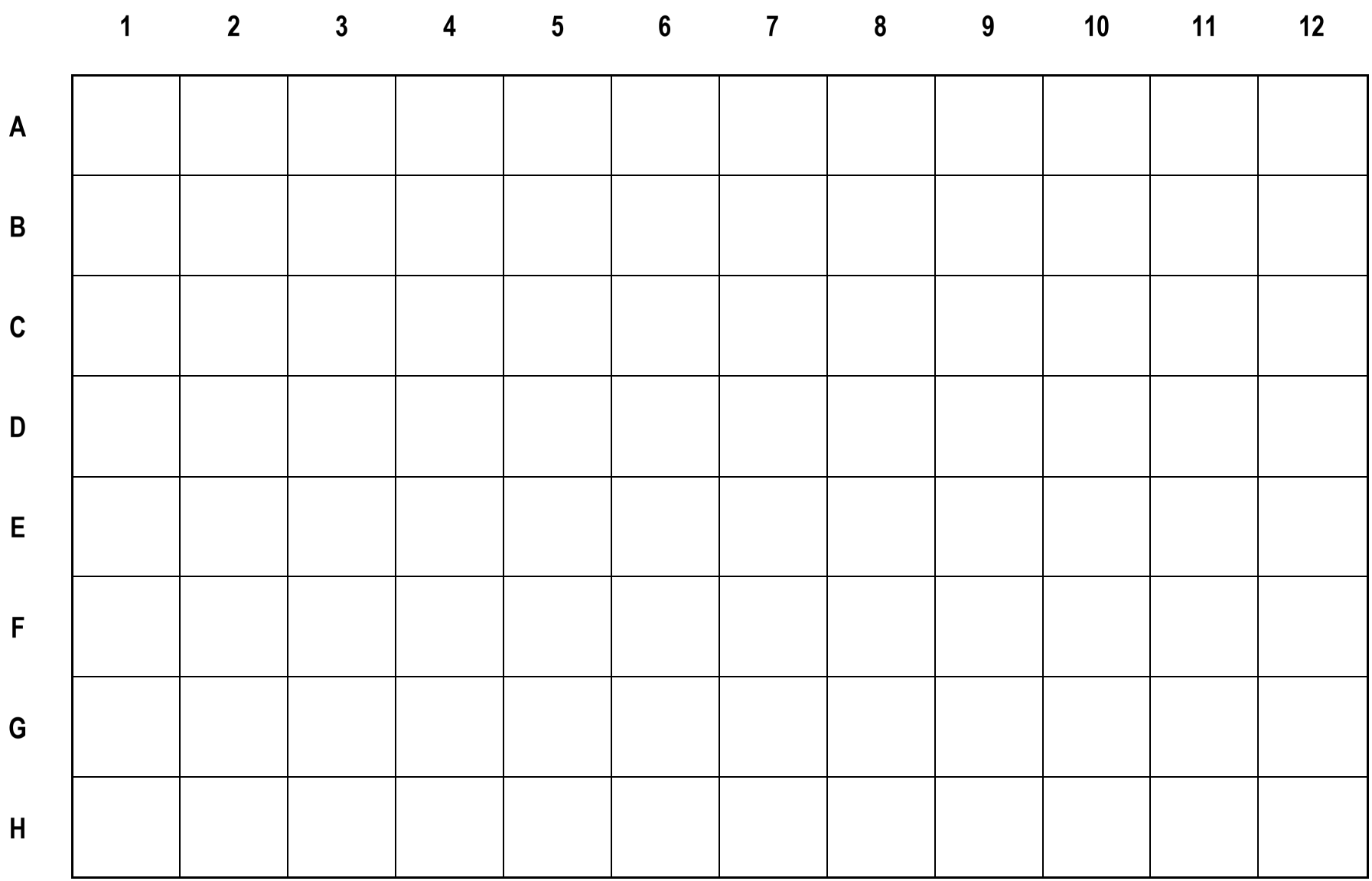
For more references on this product see our WebPages at www.biovendor.com

18. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Assay Procedure Summary

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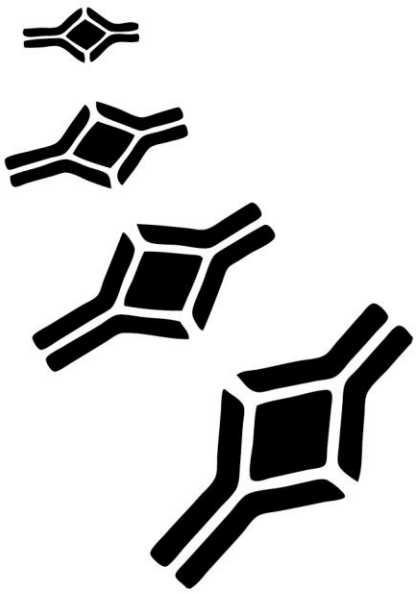

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