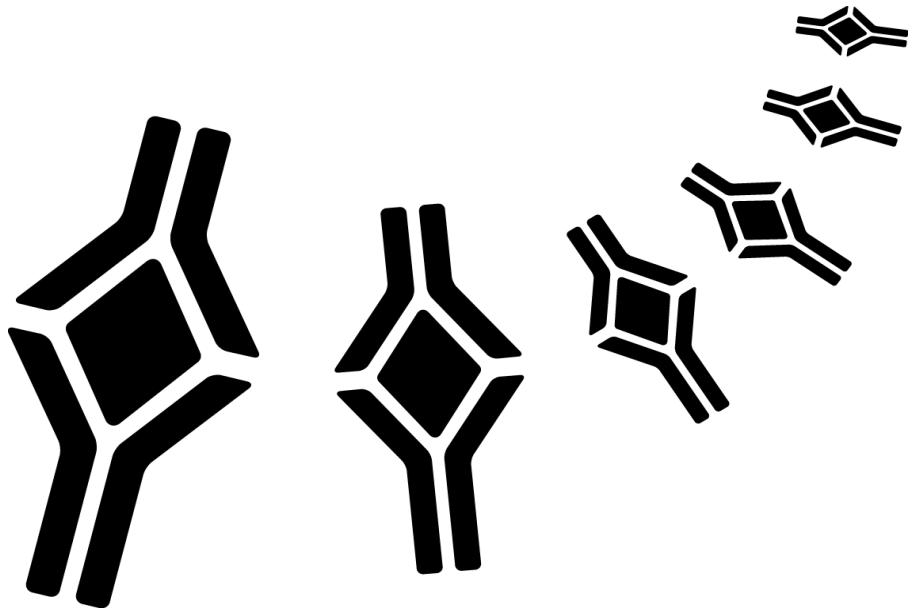
BioVendor
Research
and Diagnostic Products



HUMAN PEDF ELISA

Product Data Sheet

Cat. No.: RD191114200R

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 RD191114200R Human PEDF ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human pigment epithelium-derived factor glycoprotein (PEDF).

Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures PEDF in serum and plasma (EDTA, citrate, heparin)
- 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

PEDF was first found being synthesized and released by human fetal retinal pigment epithelial cells (RPE) into the interphotoreceptor matrix. It is localized to human chromosome 17p. PEDF is a 50 kDa multifunctional glycoprotein belonging to the serpin protease inhibitor supergene (serpin) family, acting like substrates rather than inhibitors of serine proteases, being also described as serine peptidase inhibitor, clade F (alfa-2 antiplasmin, pigment epithelium derived factor), member 1. This gene encodes a 418 amino-acid protein with an asparagine glycosylation site at position 285-287 (Asn-Leu-Thr) and N-terminal signal peptide associated with secreted proteins. PEDF has an asymmetrical charge distribution, with a high density of basic residues concentrated on one side (positive) of the molecule and of acidic residues on the opposite side. It is synthesized especially in the liver, and also in a wide range of human tissues like the lung, brain, kidney and adipose tissue.
Interactions of PEDF with three different types of molecules have been discovered: glycosaminoglycans of extracellular matrixes, collagens and receptors on the surface of neuronal cells. Negatively charged, acidic PEDF binds to collagen, lacks neurotrophic activity, and may confer antiangiogenic properties. PEDF has gliastatic, neuronotrophic, neuroprotective and antitumorigenic properties. PEDF acts in neuronal differentiation and survival in cells derived from retina and the central nervous system (CNS).Two functional epitopes have been identified on PEDF, a 34-mer peptide (residues 24–57) and a 44-mer peptide (residues 58–101). 44-mer peptide interacts with a putative 80 kDa receptor (PEDF-R^N), identified on Y-79 cells (retinoblastoma cells), cerebellar and motor neurons, and in neural retina and replicates the neurotrophic function and the ability to block vascular leackage. The 34-mer peptide, possibly via a distinct receptor (PEDF-R^A) identified on endothelial cells, induces apoptosis, blocks endothelial cell migration and corneal angiogenesis, but fails to induce Y-79 differentiation.
Recently, PEDF was shown also to have potent anti-angiogenic activity as it specifically inhibited the migration of endothelial cells, an essential step in angiogenesis. Its activity equals or supersedes that of other anti-angiogenic factors, including angiostatin, endostatin and thrombospondin-1. In cell culture and in animal models**,** PEDF inhibited endothelial cell (EC) growth and migration and suppressed ischemia-induced neovascularization, whereas in porcine liver, the expression of PEDF has been associated with body muscularity and obesity.
Analyses revealed that human PEDF is correlated with BMI, CRP, diastolic blood pressure, insulin, Quicki. Individuals with metabolic syndrome (NCEP criterion) have significantly higher PEDF values than healthy subjects, suggesting that PEDF is and independent marker of MS with sufficient diagnostic efficacy.

<u>Areas of investigation:</u>
Metabolic diseases

4. TEST PRINCIPLE

In the BioVendor Human PEDF ELISA, standards, quality controls and samples are incubated in microtitration wells coated with polyclonal anti-human PEDF antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human PEDF antibody is added and incubated with captured PEDF for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 60 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 PEDF. A standard curve is constructed by plotting absorbance values against 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. 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. However, 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	ready to use	13 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	75 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 5-1 000 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 550-650nm)
- 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.

Biotin Labelled Antibody
Streptavidin-HRP Conjugate
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 PEDF 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 PEDF in the stock solution is **6 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	6 ng/ml
250 µl of stock	250 µl	3 ng/ml
250 µl of 3 ng/ml	250 µl	1.5 ng/ml
250 µl of 1.5 ng/ml	375 µl	0.6 ng/ml
250 µl of 0.6 ng/ml	250 µl	0.3 ng/ml
250 µl of 0.3 ng/ml	250 µl	0.15 ng/ml

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

Stability and storage:

Standard stock solution 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).

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

Stability and storage:

The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

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 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 PEDF in serum and plasma (EDTA, citrate, heparin).

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.

Dilute samples just prior to the assay 8 000x with Dilution Buffer, in two steps as follows:
Dilution A (100x):
Add 5 µl of sample into 495 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.
Dilution B (80x):
Add 5 µl of Dilution A into 395 µl of Dilution Buffer to prepare final dilution (8 000x) and **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 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 PEDF.

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 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 Labelled 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 **1 hour**, 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 **5 minutes** at room temperature. The incubation time may be extended [up to 10 - 15 minutes] if the reaction temperature is below than 20°C. Solution in wells will turn blue. 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 PEDF 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 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 6**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 3**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 1.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 0.6**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.3**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.15**	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 log of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of PEDF (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. 3.5 ng/ml (from standard curve) x 8 000 (dilution factor) = 28 μg/ml.

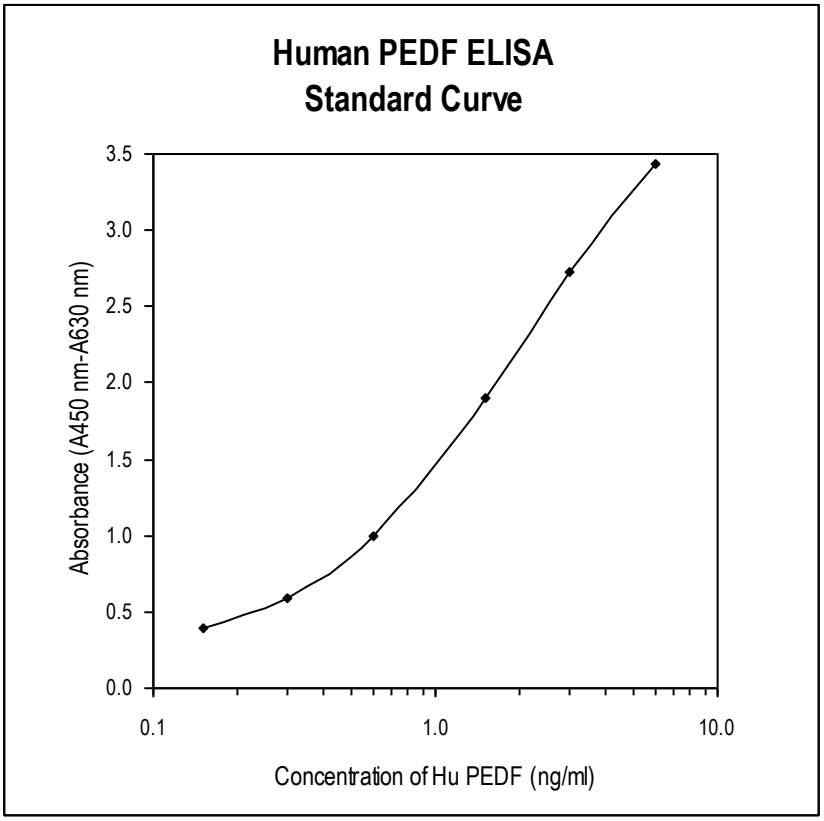


Figure 2: Typical Standard Curve for Human PEDF ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human PEDF 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 PEDF values in wells and is 0.045 ng/ml
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding PEDF level of 48 μg/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the PEDF concentration.

- **Specificity**

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

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	yes
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 (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	4.09	0.12	2.92
2	5.60	0.23	4.05
3	6.61	0.25	3.73

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	3.32	0.19	5.9
2	2.21	0.12	5.3
3	0.29	0.02	6.6

- **Spiking Recovery**

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

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	2.00	-	-
	8.82	9.32	94.6
	5.68	5.60	101.4
	3.88	3.72	104.3
2	1.32	-	-
	8.44	8.64	97.7
	5.02	4.92	102.0
	3.14	3.04	103.3

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved* (*µg/ml*)	***E**xpected* (*µg/ml*)	*Recovery* ***O/E** (%)*
1	-	11.26	-	-
	2x	5.65	5.63	100.4
	4x	2.64	2.82	93.9
	8x	1.44	1.41	102.3
2	-	16.15	-	-
	2x	8.04	8.08	99.6
	4x	3.74	4.04	92.5
	8x	1.92	2.02	95.3

- **Effect of sample matrix**

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

Volunteer No.	*Serum (µg/ml)*	*Plasma (µg/ml)*		
		EDTA	*Citrate*	*Heparin*
1	12.5	11.7	11.1	14.6
2	19.0	19.6	15.1	16.1
3	15.5	15.6	12.0	14.6
4	14.8	13.8	11.8	15.7
5	13.4	10.7	10.9	13.8
6	13.0	10.2	11.5	14.9
7	11.8	12.0	10.2	11.0
8	10.4	10.4	8.4	12.7
9	13.3	13.9	11.5	15.3
10	13.9	17.3	10.9	13.3
Mean (µg/ml)	**13.89**	**13.72**	**11.36**	**14.14**
Mean Plasma/Serum (%)	**-**	**98.0**	**82.8**	**104.5**

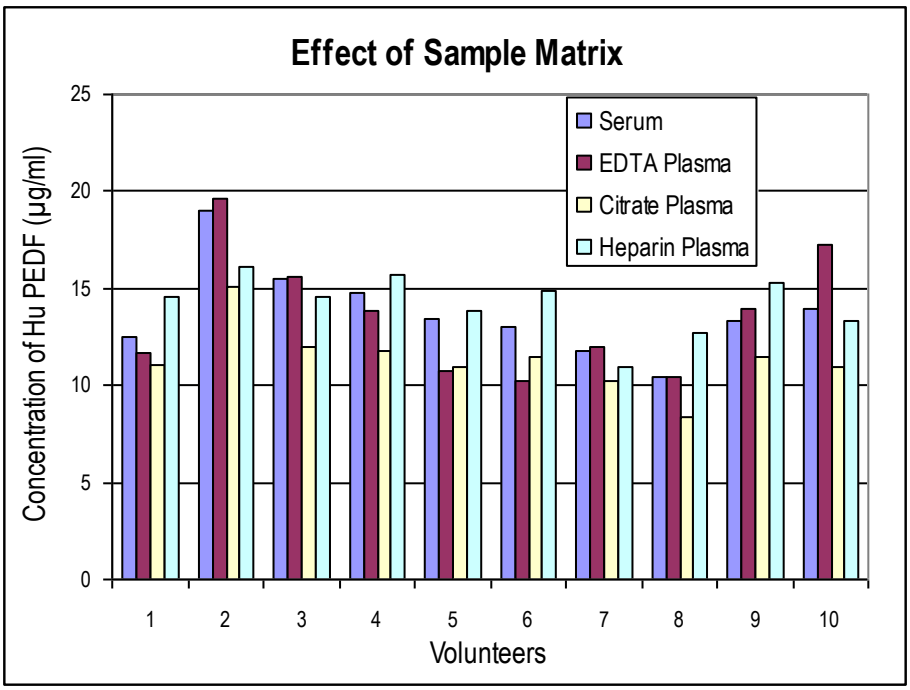


Figure 3: PEDF levels measured using Human PEDF ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at –20°C. However, no decline in concentration of PEDF 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 (µg/ml)*	*Plasma (µg/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	15.53	13.04	11.90	12.20
	2-8°C, 1 day	13.24	11.60	10.16	10.23
	2-8°C, 7 days	14.00	13.85	10.55	12.54
2	-20°C	9.66	9.92	6.84	8.75
	2-8°C, 1 day	9.96	8.68	6.63	9.48
	2-8°C, 7 days	8.83	8.32	7.00	8.28
3	-20°C	12.85	12.42	10.88	11.64
	2-8°C, 1 day	11.68	11.28	9.58	11.42
	2-8°C, 7 days	14.12	12.37	9.89	12.96

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human PEDF 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 (µg/ml)*	*Plasma (µg/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	9.84	11.03	9.92	9.96
	3x	13.17	10.70	8.30	10.66
	5x	10.98	12.72	9.19	9.92
2	1x	11.86	11.96	10.20	11.02
	3x	12.32	10.81	9.82	12.74
	5x	14.99	14.70	8.40	10.72
3	1x	9.03	9.32	8.08	9.26
	3x	10.52	8.23	8.36	8.57
	5x	10.89	10.02	9.06	9.71

14. DEFINITION OF THE STANDARD

The recombinant human PEDF is used as the Standard. The recombinant human PEDF, produced in *E. coli*, is 46.1 kDa protein containing 413 amino acid residues of the human PEDF and 14 additional amino acid residues – His Tag.

15. PRELIMINARY POPULATION AND CLINICAL DATA

Sera from 112 patients with metabolic syndrome and without components of metabolic syndrome were measured. Men and women did not differ in PEDF serum values.
PEDF correlated with BMI (r=0.32, P<0.01), CRP (r=0.33, P<0.01), diastolic blood pressure =(r=0.3, P<0.01), insulin (0.82, P=0.02), Quicki (r=-0.22, P=0.048).
Individuals with metabolic syndrome (NCEP criteria) had significantly higher PEDF values (medians 15.6 µg/ml vs. 11.2 µg/ml; P <0.01) than healthy subjects.

- **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 references ranges for PEDF levels with the assay.

16. METHOD COMPARISON

The Biovendor's Human PEDF 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, Quality Controls or samples

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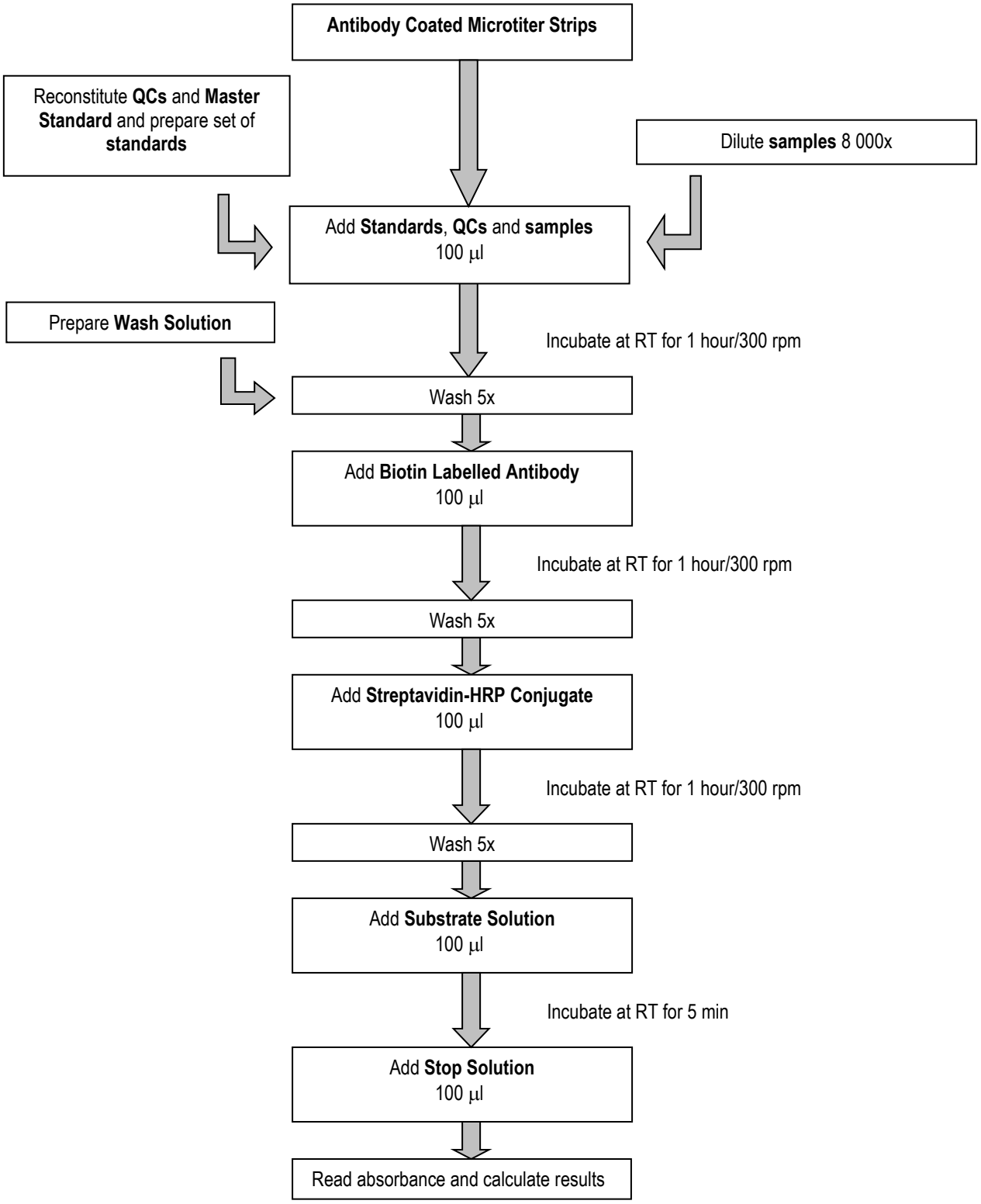
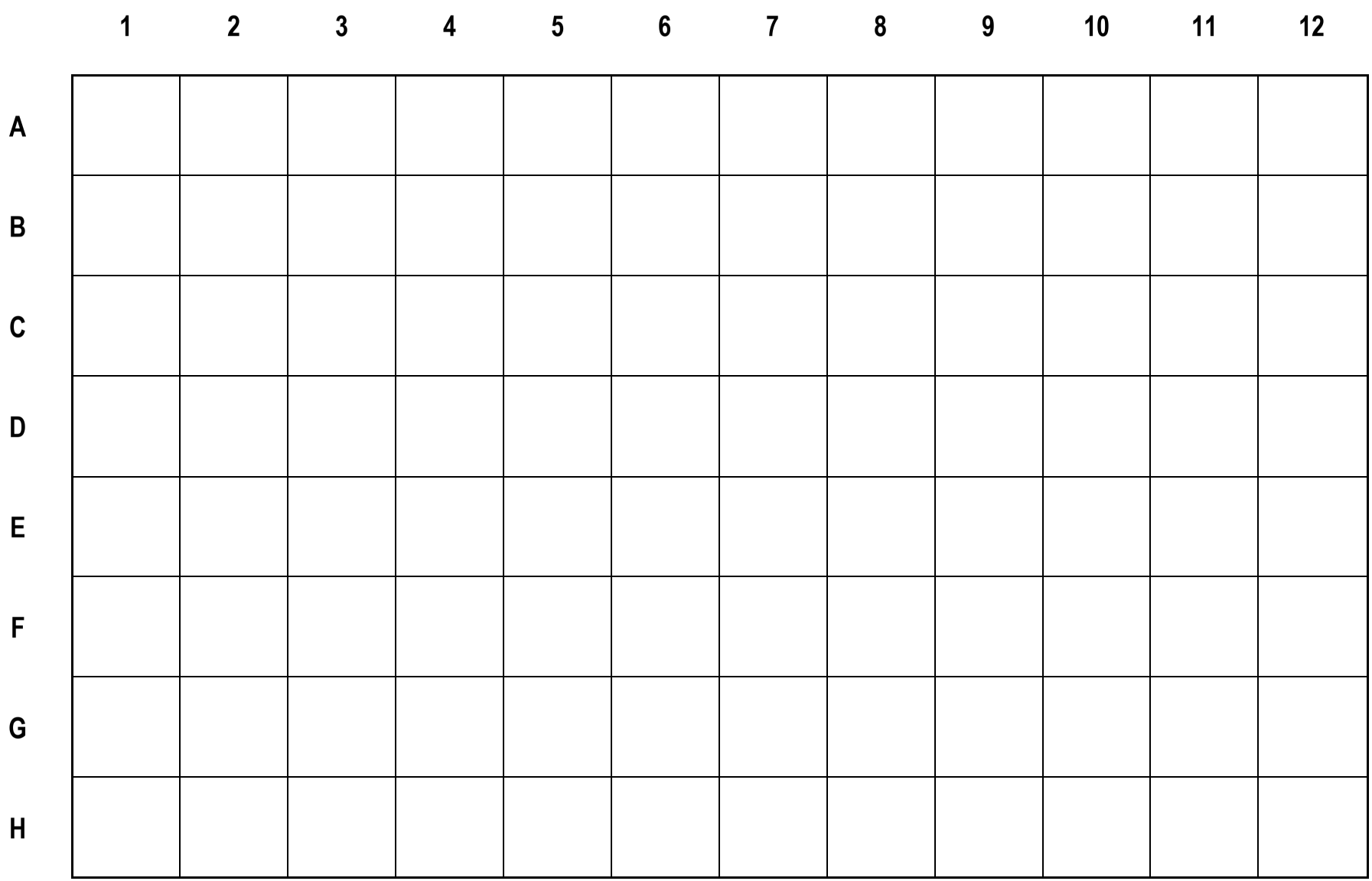
19. EXPLANATION OF SYMBOLS

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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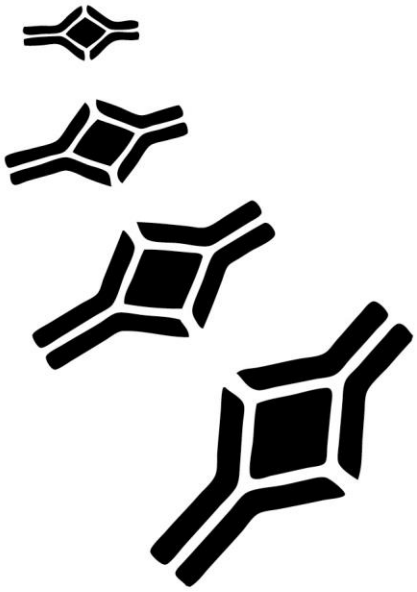

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