BioVendor Group

ENG

Instructions for Use:

HUMAN PENTRAXIN 3 ELISA

Catalogue number:

RD191477200R

European Union:

IVD CE

Rest of the world:

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.006.A	ENG.007.A
Product Data Sheet	Instructions for Use
History of changes" added.	
Chapter 9: A sentence „Centrifuge liquid containing microtube vials before opening" added.	
Symbol indicating the manufacturer added.	
Chapter 19 Additional information added.	

1. INTENDED USE

The RD191477200R Human Pentraxin 3 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human pentraxin 3 (PTX3).

Features

- **European Union: for in vitro diagnostic use**
- **Rest of the world: for research use only!**
- The total assay time is less than 3.5 hours
- The kit measures PTX3 in serum, plasma (EDTA, citrate, heparin), saliva and stool extract
- For protocol for preparation of stool extracts and other details, please contact us at info@biovendor.com
- 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

Pentraxin 3 (PTX3, TSG14, Tumor necrosis factor alpha-induced protein 5, Tumor necrosis factor-inducible gene 14 protein) is an evolutionarily conserved, multimeric acute phase inflammatory glycoprotein in the same family as the well-established cardiovascular biomarker C-reactive protein (CRP). It is the prototypical long pentraxin, exhibiting a C-terminal pentraxin domain characteristic of the family, and a unique N-terminal domain [1,13].
PTX3 is mainly produced at extrahepatic sites by several cell types, including cells of the myelomonocyte lineage (monocytes, macrophages, dendritic cells), endothelial cells, smooth muscle cells, fibroblasts, and adipocytes. PTX3 is also produced during neutrophil differentiation and stored in specific granules of mature neutrophils, ready to be released upon microbial recognition [13].
PTX3 behaves as an acute phase response protein, as the blood levels of PTX3, low in normal conditions (<2 ng/ml in humans), increase rapidly (peaking at 6-8 hours after induction) and

dramatically (200-800 ng/ml) during endotoxic shock, sepsis, and other inflammatory and infectious conditions, correlating with the severity of the disease.
PTX3 has multiple complex nonredundant functions, ranging from assembly of a hyaluronic acid–rich extracellular matrix and female fertility to protection against pathogens (i.e., ***Aspergillus fumigatus***, influenza viruses). PTX3 also regulates the clearance of apoptotic cells and may participate in maintenance of immunologic tolerance [13].
PTX3 is expressed in response to proinflammatory signals, including bacteria, IL-1 (but not IL-6), and TN-alpha produced primarily by endothelial cells, neutrophils, and macrophages. As a result, inflammation diseases, especially disorders of the immune system such as rheumatoid arthritis, progressive systemic sclerosis, Churg-Straus syndrome, Wegener's granulomatosis, and microscopic polyangiitis, as well as systemic inflammatory response syndrome (SIRS), result in increased expression of plasma PTX3 [11]. PTX3 is elevated in critically ill patients, with a gradient from systematic inflammatory response syndrome to septic shock, and in several other diseases, such as myocardial infarction, atherosclerosis, small vessel vasculitis and psoriasis [9,11].
Plasma PTX3 levels have also been suggested to be a good marker for the response to treatment of patients with obstructive sleep apnea (OSA) [12].
PTX3 may also be a good diagnostic marker for deterioration in patients with inflammatory bowel disease [11,16].
Pentraxin 3 has been proposed as a marker of endothelial dysfunction and inflammation in pre-eclampsia. Pentraxin 3 levels have been found to be elevated in normal pregnancy and also shown to be significantly higher at the time of diagnosis of pre-eclampsia when compared with normal pregnancy [15].
PTX3 may also be elevated in malignancies such as lung carcinoma, prostate cancer and liposarcomas [7,10].

Areas of investigation:
Cardiovascular disease
Immune response
Infection and Inflammation
Inflammatory bowel disease
Sepsis
Oncology

4. TEST PRINCIPLE

In the BioVendor Human Pentraxin 3 ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human PTX3 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human PTX3 antibody is added and incubated for 60 minutes with captured human PTX3. 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 PTX3. 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 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 Conc. (100x)	concentrated	0.13 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	20 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

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution
- 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.

Centrifuge liquid containing microtube vials before opening.

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

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

Human PTX3 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 PTX3 in the stock solution is **5000 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	5000 pg/ml
250 µl of stock	250 µl	2500 pg/ml
250 µl of 2500 pg/ml	250 µl	1250 pg/ml
250 µl of 1250 pg/ml	250 µl	625 pg/ml
250 µl of 625 pg/ml	250 µl	313 pg/ml
250 µl of 313 pg/ml	250 µl	156 pg/ml
250 µl of 156 pg/ml	250 µl	78 pg/ml

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

Stability and storage:

Do not store the reconstituted Master Standard and/or 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 IFU and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Conc. (100x) to 99 parts Biotin-Ab Diluent.
Example: 10 µl of Biotin Labelled Antibody Conc. (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells). **Mix well** (not to foam).

Stability and storage:
Opened Biotin Labelled Antibody Conc. (100x) 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 Conc. (10x) ten-fold in distilled water to prepare a 1x working solution. e.g. 100 ml of Wash Solution Conc. (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 Conc. (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures PTX3 in serum, plasma (EDTA, citrate, heparin), saliva and stool extract

Samples can be assayed immediately after collection, or after long-term storage at -20°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.

An appropriate dilution should be assessed by the researched prior to batch measurement, with respect to large variability in PTX3 serum levels (healthy individuals exhibit low PTX3 levels while during sepsis, PTX3 levels increase profoundly). Recommended starting dilution for samples from healthy donors is 3-fold, recommended starting dilution for samples from patients with sepsis is 200-fold.

Serum, plasma and saliva samples:

Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 80 µl of sample + 160 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°for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

Stool extract:

For protocol for preparation of stool extracts and other details, please contact us at info@biovendor.com.
Recommended starting dilution for stool extract is 3x.

Stability and storage:
Stool samples should be stored at 2-8°C for up to 6 days. For long-term storage, they should be stored at -20°C, or preferably at -70°C. Extracts should be storage at -20°C, or preferably at -70°C for at least 4 months. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

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

11. ASSAY PROCEDURE

1. Pipet **100 µl** of 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Pipet **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. Pipet **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 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 the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine PTX3 concentration of off-scale samples. The readings at 405 nm should not replace the reading 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 5000**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 2500**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 1250**	Sample 1	Sample 9	Sample 17	Sample 22	Sample 33
D	**Standard 625**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 313**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 156**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 78**	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 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 PTX3 (pg/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. 500 pg/ml (from standard curve) x 3 (dilution factor) = 1500 pg/ml.

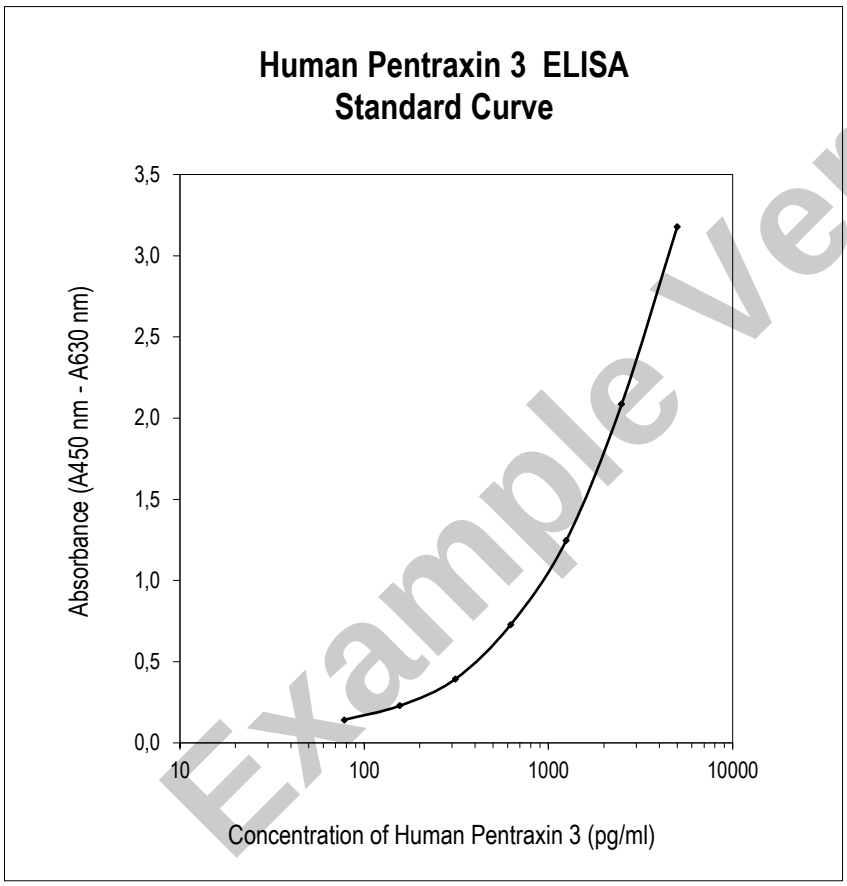

Figure 2: Typical Standard Curve for Human Pentraxin 3 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Pentraxin 3 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 PTX3 values in wells and is 22 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 Pentraxin 3.
We observed no interference of hemoglobin (1.0 mg/ml), bilirubin (170 µmol/l) and triglycerides (5.0 mmol/l).
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 (pg/ml)	SD (pg/ml)	CV (%)
1	2708	105	3.9
2	4500	128	2.9

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

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	1026	86	8.4
2	1808	108	6.0

Spiking Recovery

Serum samples were spiked with different amounts of human PTX3 and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	660 2305 1568 1010	- 2535 1599 1128	- 90.9 98.1 89.5
2	642 2415 1383 1047	- 2517 1581 1110	- 95.9 87.6 94.4

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	- 2x 4x 8x	3212 1474 746 406	- 1606 803 401	- 91.8 92.9 101.2
2	- 2x 4x 8x	3315 1590 852 411	- 1658 829 414	- 95.9 102.8 99.2

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 (pg/ml)	Plasma (pg/ml)		
		EDTA	Citrate	Heparin
1	1535	1529	1260	1226
2	3305	3948	3150	4234
3	1986	1702	1334	2467
4	1870	1570	1200	2743
5	2136	2294	1515	2066
6	2552	2872	2215	2704
7	1416	1651	1031	2066
8	1599	1565	1052	1283
9	1584	1267	1113	1634
10	1646	1647	1111	1342
Mean (pg/ml)	**1963**	**2004**	**1498**	**2176**
Mean Plasma/Serum (%)		**102.1**	**76.3**	**110.9**
Coefficient of Determination R^2	**-**	**0.93**	**0.95**	**0.78**

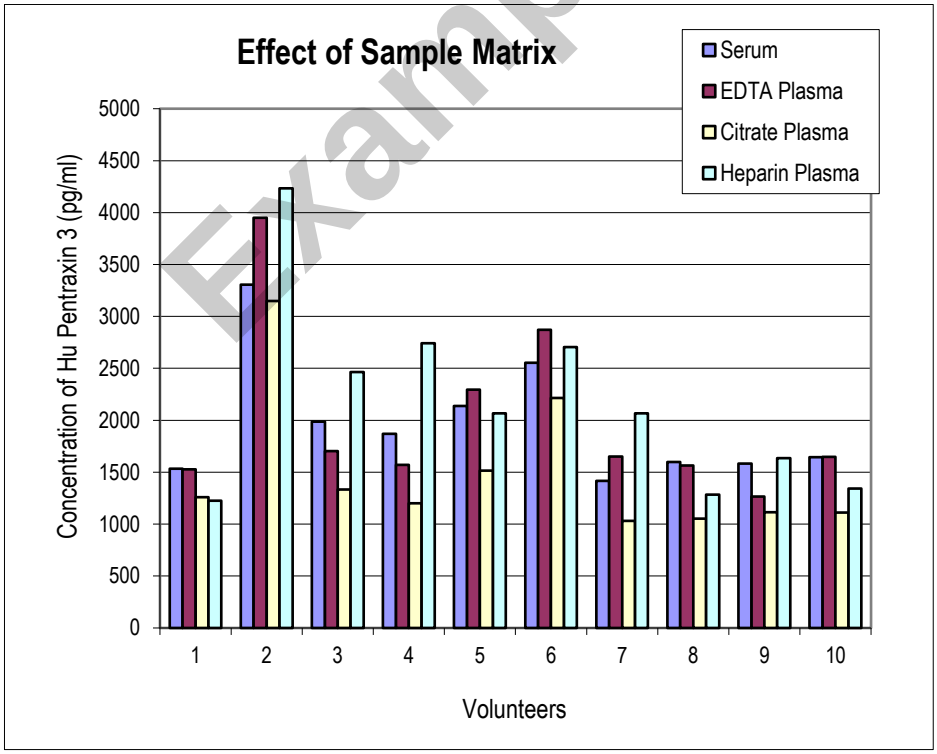

Figure 3: PTX3 levels measured using Human Pentraxin 3 ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

Stability of samples stored at 2-8°C

Samples should be stored at –20°C. However, no decline in concentration of PTX3 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 thimerosal, resulting in the final concentration of 0.03% and 0.01%, respectively.

Sample	Incubation Temp, Period	Serum (pg/ml)	Plasma (pg/ml)		
			EDTA	Citrate	Heparin
1	-20°C	1619	3028	1511	1883
	2-8°C, 1 day	1726	3615	1710	2078
	2-8°C, 7 days	1597	2706	1392	1716
2	-20°C	581	1091	516	1079
	2-8°C, 1 day	562	895	597	881
	2-8°C, 7 days	494	956	435	844
3	-20°C	1873	4311	2025	2695
	2-8°C, 1 day	1893	4741	1597	2546
	2-8°C, 7 days	1684	4717	1572	2185

Effect of Freezing/Thawing

No decline was observed in concentration of human PTX3 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 (pg/ml)	Plasma (pg/ml)		
			EDTA	Citrate	Heparin
1	1x	962	1433	925	986
	3x	1046	1674	804	917
	5x	838	1296	780	1069
2	1x	1101	2335	906	1312
	3x	884	1964	858	1291
	5x	908	1946	831	1198
3	1x	1537	3127	1702	2084
	3x	1684	3090	1479	2117
	5x	1256	2688	1429	1713

14. DEFINITION OF THE STANDARD

Recombinant Human Pentraxin 3, expressed in HEK293, is used as the standard. The protein has 370 amino acids and molecular weight 41 kDa.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 116 unselected donors (70 men + 46 women) 21 – 65 years old were assayed with the Biovendor Human Pentraxin 3 ELISA in our laboratory.

Sex	Age (years)	n	Mean	Median	SD	Min	Max
			PTX3 (pg/ml)				
Men	21-29	13	1582	1199	891	700	4081
	30-39	18	1299	1060	538	716	2679
	40-49	27	1302	1104	536	620	3332
	50-65	12	1127	1154	255	721	1558
Women	22-29	11	1934	1826	451	1313	2626
	30-39	16	1462	1304	615	711	3207
	40-49	17	1122	1110	380	626	2137
	50-61	2	1626	1626	894	733	2519

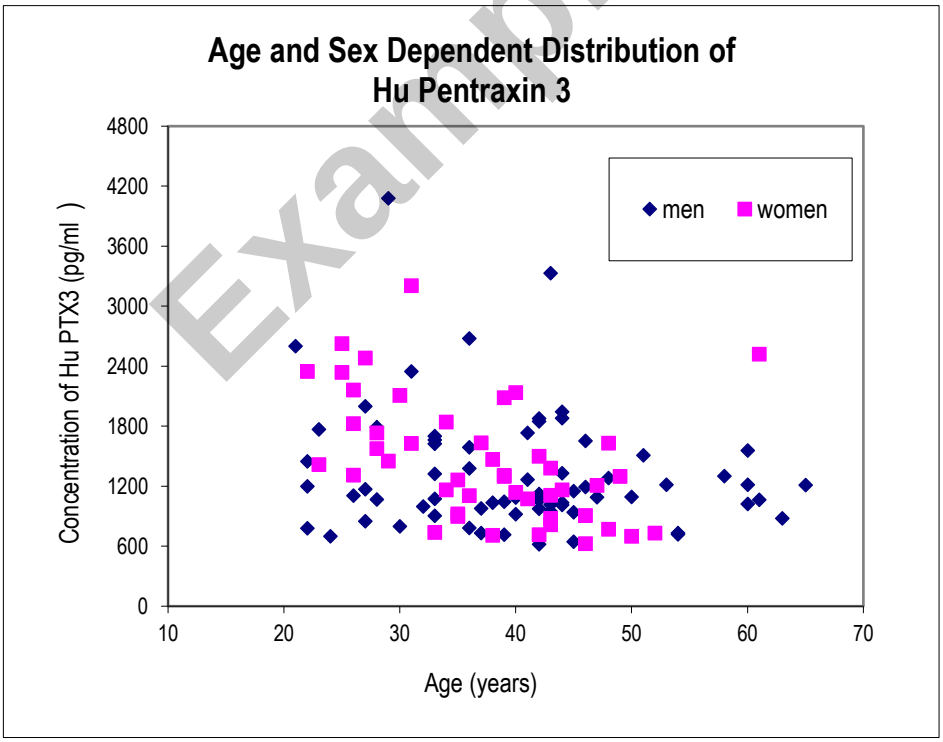

Figure 4: Human PTX3 concentration plotted against donor age and sex.

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 samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for human PTX3 protein levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Pentraxin 3 ELISA has not been compared to any other commercial 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 manual washing
- 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

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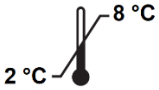
For more references on this product see our web pages at www.biovendor.com.

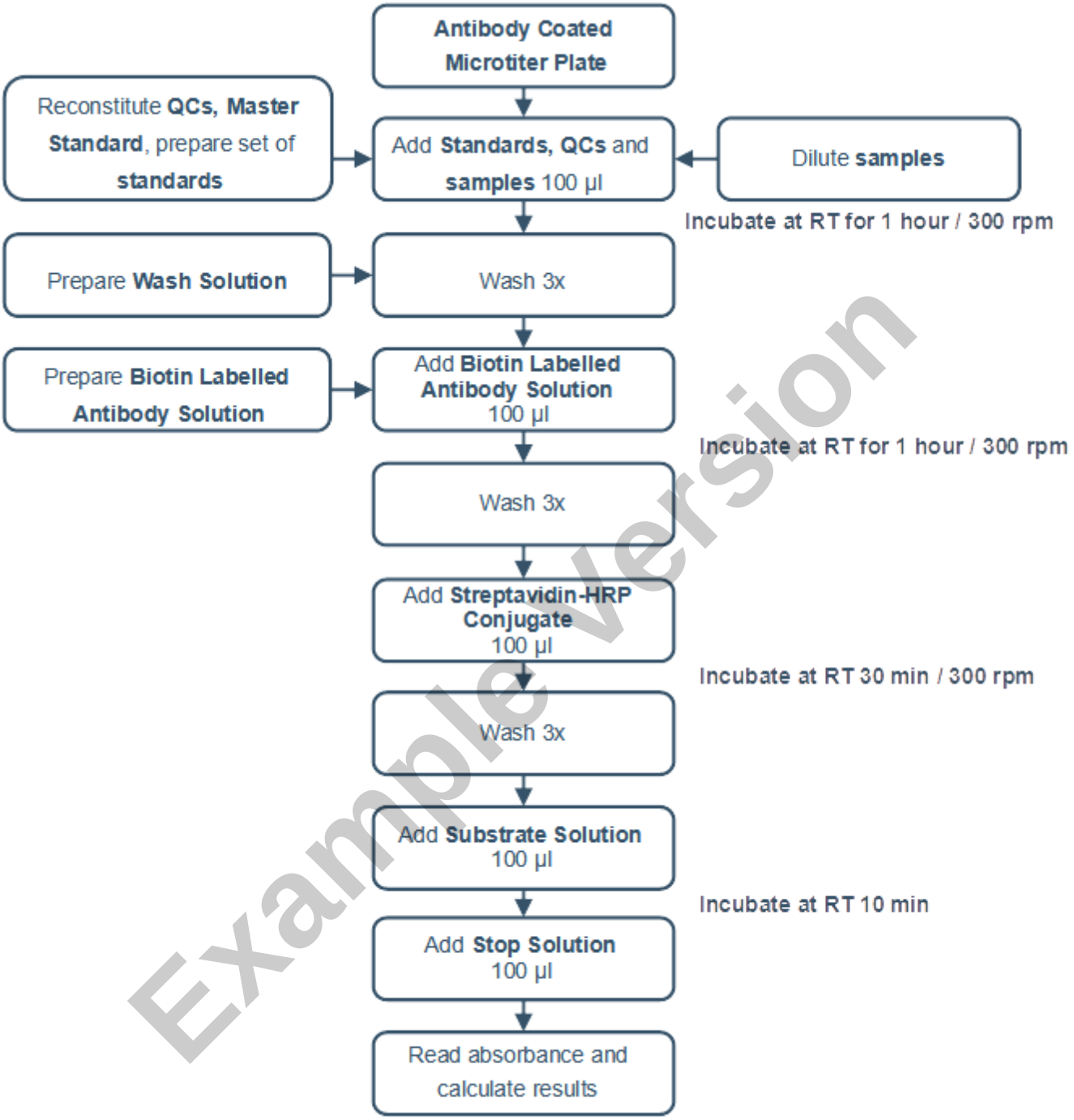
19. ADDITIONAL INFORMATION

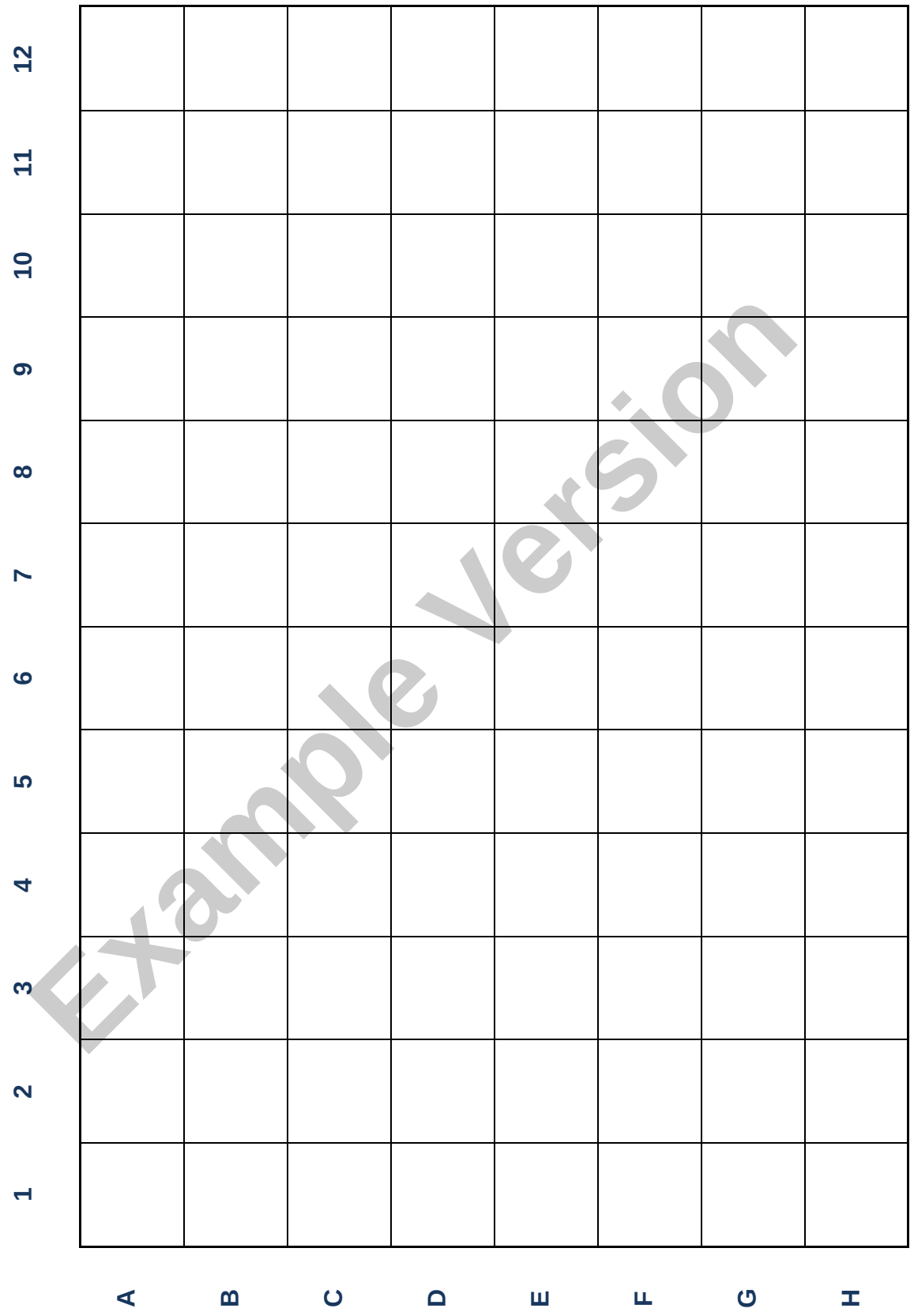
Any serious incident occurring in connection with the device must be reported to the manufacturer and to the competent authority of the Member State in which the user or patient is located.

20. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks
	In vitro diagnostic medical device
	CE marking of conformity

21. ASSAY PROCEDURE - SUMMARY





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