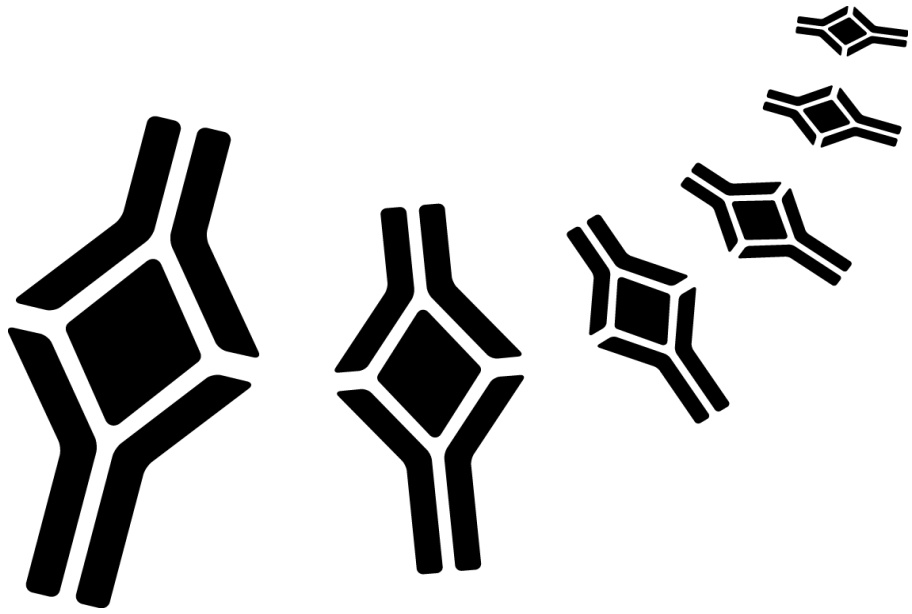
BioVendor
Research
and Diagnostic Products



HUMAN TIMP-1 ELISA

Product Data Sheet

Cat. No.: RD191511100R

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 RD191511100R Human TIMP-1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human tissue inhibitor of metalloproteinase 1 (TIMP-1).

Features

- **It is intended for research use only**
- The total assay time is less than 2.5 hours
- The kit measures TIMP-1 in serum, plasma (EDTA, citrate, heparine), saliva and cerebrospinal fluid (CSF)
- 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

Tissue inhibitor of metalloproteinase 1 (TIMP-1), located on chromosome Xp11.3-p11.23, belongs to the Tissue Inhibitor of Metalloproteinases family which includes four identified members (TIMP-1, TIMP-2, TIMP-3, and TIMP-4). TIMP-1 encodes a 931 base-pair mRNA and a 207 amino acid protein [1]. Studies have shown that this protein may inhibit the proteolytic activity of matrix metalloproteinases (MMPs) by forming noncovalent 1:1 stoichiometric complexes and regulates the balance of matrix remodeling during degradation of extracellular matrix [2].

TIMP-1 has been implicated in a number of other biological processes, including growth factor activity, tissue remodeling, inhibition of angiogenesis, changes in cell morphology, and stimulation of gonadal steroidogenesis. In the CNS, TIMP-1 provides neuroprotective effects through its role in blood–brain barrier maintenance, which is accomplished by interacting with ECM components, inhibiting MMPs, and reducing glutamate-mediated calcium influx following excitotoxic stress. In the context of brain pathogenesis, TIMP-1 is linked to diseases such as multiple sclerosis, Parkinson's disease and human brain tumors [3].

Recently, clinical studies have shown that the aberrant expression of TIMP-1 is associated with an unfavorable prognosis in a series of tumors, such as gastric cancer [4], papillary thyroid carcinoma [5], cutaneous melanoma [6], breast cancer [7] lung and colorectal cancers [8,1]. Interestingly, in severe sepsis, the expression level of TIMP-1 is significantly elevated. MMP-2, MMP-9, TIMP-1, TIMP-2 and IL-6 plasma levels were measured in patients with severe sepsis and TIMP-1 showed the highest sensitivity, specificity, and positive predictive value for sepsis prognosis, confirming TIMP-1 as a predictor of clinical outcome in patients with severe sepsis [9].

Matrix metalloproteinases are indispensable elements of tissue reconstruction. MMPs released from macrophages lead to the destruction of elastin and cause emphysema. These enzymes participate in normal body functions but they behave differently in chronic obstructive pulmonary disease (COPD). The genetic polymorphisms of TIMP-1 and TIMP-2 are the tissue inhibitors of MMPs that have been found to be associated with COPD [10]. Another recent study demonstrated that TIMP-1 is present at high levels in sera from patients with tuberculosis (TB), and that expression of TIMP-1 mRNA is induced by mycobacteria. TIMP-1 may therefore be a potential biomarker of tuberculosis in humans [11].

<u>Areas of investigation:</u>
Extracellular matrix
Oncology
Sepsis

4. TEST PRINCIPLE

In the BioVendor Human TIMP-1 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human TIMP-1 antibody. After 60 minutes incubation and washing, HRP labelled polyclonal anti-human TIMP-1 antibody is added and incubated for 60 minutes with captured TIMP-1. 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 TIMP-1. 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 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 been 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 Conc. (100x)	concentrated	0.13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	2x20 ml
Conjugate Diluent	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precise 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 shaking at 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Conjugate 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 TIMP-1 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 human TIMP-1 in the stock solution is **16 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	16 ng/ml
250 µl of stock	250 µl	8 ng/ml
250 µl of 8 ng/ml	250 µl	4 ng/ml
250 µl of 4 ng/ml	250 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml

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

Stability and storage:
Do not store the reconstituted Master Standard and/or diluted standard solutions.

Conjugate Solution Conc. (100x)
Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Conc. (100x) to 99 parts Conjugate Diluent. Example: 10 µl of Conjugate Solution Conc. (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells).
Stability and storage:
Opened Conjugate Solution Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Conjugate Solution Conc. (100x).

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 TIMP-1 in serum, plasma (EDTA, citrate, heparin), saliva and cerebrospinal fluid

Samples should be assayed immediately after collection or should be stored 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 researcher in advance to batch measurement.

Recommended starting dilution for serum, plasma and saliva samples is 100x.
Dilute samples just prior to perform the test **100x** with Dilution Buffer in two steps as follows:
Dilution A (10x):
Add 5 µl of sample into 45 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.
Dilution B (10x):
Add 24 µl of Dilution A into 216 µl of Dilution Buffer to prepare final dilution 100x. **Mix well** (not to foam). Vortex is recommended.

Recommended starting dilution for cerebrospinal fluid samples is 25x.

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C or lower 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 human Timp-1.

Note: It is recommended to use a precise 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, 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 working 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 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 12.**

Note 1: 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 TIMP-1 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 16**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 8**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 4**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 2**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 1**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 0.5**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 34
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 TIMP-1 (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. 10 ng/ml (from standard curve) x 100 (dilution factor) = 1000 ng/ml.

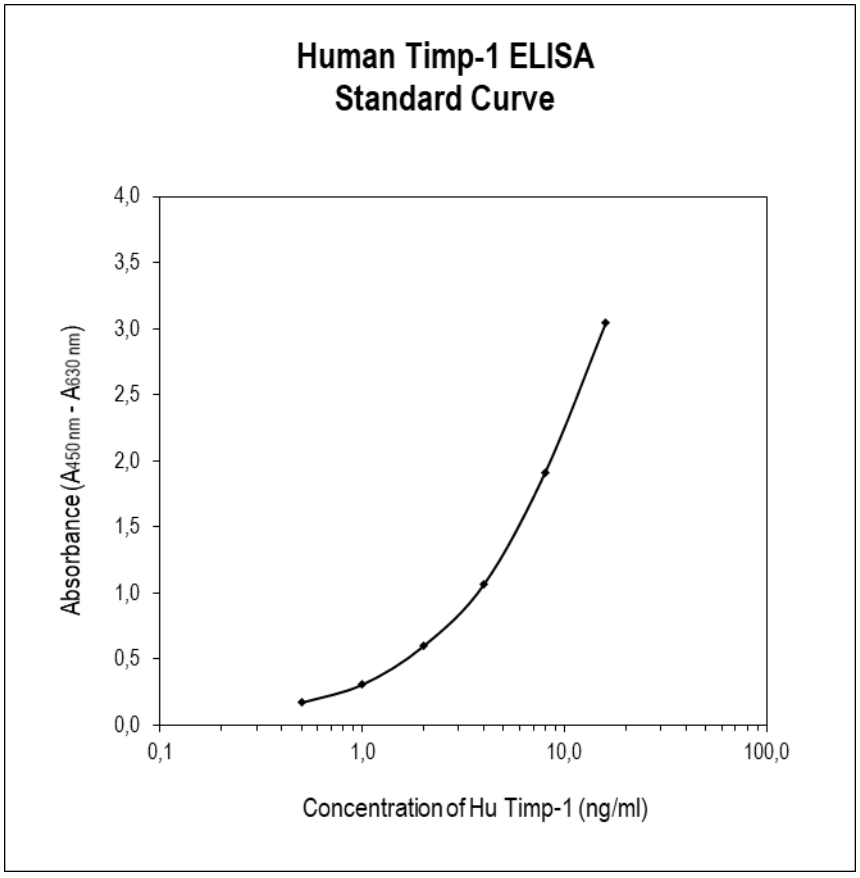


Figure 2: Typical Standard Curve for Human TIMP-1 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human TIMP-1 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 prorenin values in wells and is 0.032 ng/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.

Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
Serum 1	355.49	11.68	3.3
Serum 2	91.49	6.14	6.7

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
Serum 1	175.60	10.03	5.7
Serum 2	205.28	17.21	8.4

- **Spiking Recovery**

Samples were spiked with different amounts of human TIMP-1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	108.00	-	-
	263.70	258.0	93.8
	411.60	408.0	100.9
	664.20	708.0	102.2
Serum 2	148.50	-	-
	322.20	298.5	89.2
	466.05	448.5	103.9
	667.50	748.5	107.9

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	-	154.70	-	-
	2x	75.98	77.35	98.2
	4x	37.50	38.68	97.0
	8x	17.05	19.34	88.2
Serum 2	-	151.83	-	-
	2x	74.98	75.91	98.8
	4x	39.83	37.96	104.9
	8x	19.18	18.98	101.0

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. A weak correlation was observed between serum and plasma EDTA, citrate and plasma heparin samples. Results are shown below:

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	186.70	134.9	78.4	126.0
2	239.30	127.0	81.8	205.4
3	195.80	109.0	64.6	84.1
4	219.10	113.9	92.5	121.1
5	201.20	91.1	72.9	127.3
6	185.20	141.9	87.5	103.5
7	256.90	94.3	63.8	82.6
8	174.20	137.8	85.0	103.2
9	163.10	109.6	69.0	83.2
10	199.40	107.6	82.1	88.3
Mean (ng/ml)	**202.1**	**116.7**	**77.8**	**112.5**
Mean Plasma/Serum (%)	**-**	**58**	**38**	**56**
Coefficient of Determination R^2	**-**	**0.15**	**0.02**	**0.13**

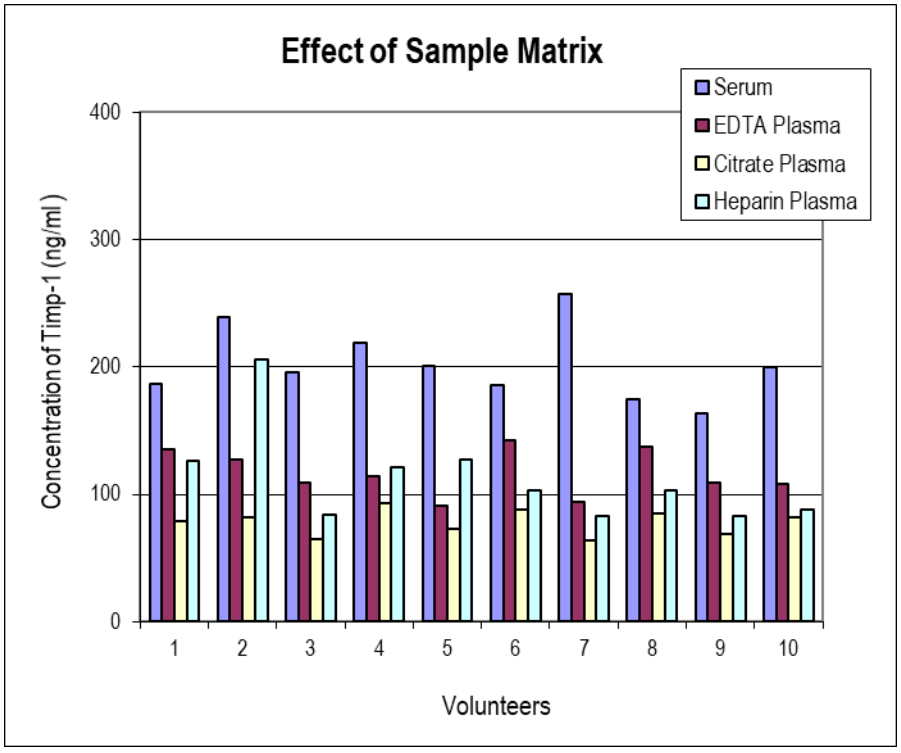


Figure 3: TIMP-1 levels measured using Human Timp-1 ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

14. DEFINITION OF THE STANDARD

Recombinant human Timp-1 is used as the standard. The recombinant human Timp-1 produced in HEK293 cells is a 21.2 kDa protein containing 190 amino acid residues of human Timp-1 and C-terminal His-tag 6 AA.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 124 unselected donors (69 men + 55 women) 21-65 years old were assayed with the BioVendor Human Timp-1 ELISA in our laboratory.

- **Age dependent distribution of Timp-1**

Sex	*Age (years)*	*n*	*Timp-1 (ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	21-29	11	124.63	137.20	60.24	1.01	179.80
	30-39	19	163.64	162,90	29.50	108.00	234.00
	40-49	28	173.55	163.50	52.24	57.00	342.00
	50-65	11	183.32	176.70	28.41	141.00	224.00
Women	22-29	11	169.50	157.60	35.86	127.10	267.10
	30-39	19	166.00	161.90	25.64	110.80	220.80
	40-49	18	158.55	156.75	41.26	53.30	267.00
	50-61	7	173.70	158.20	29.37	123.10	204.20

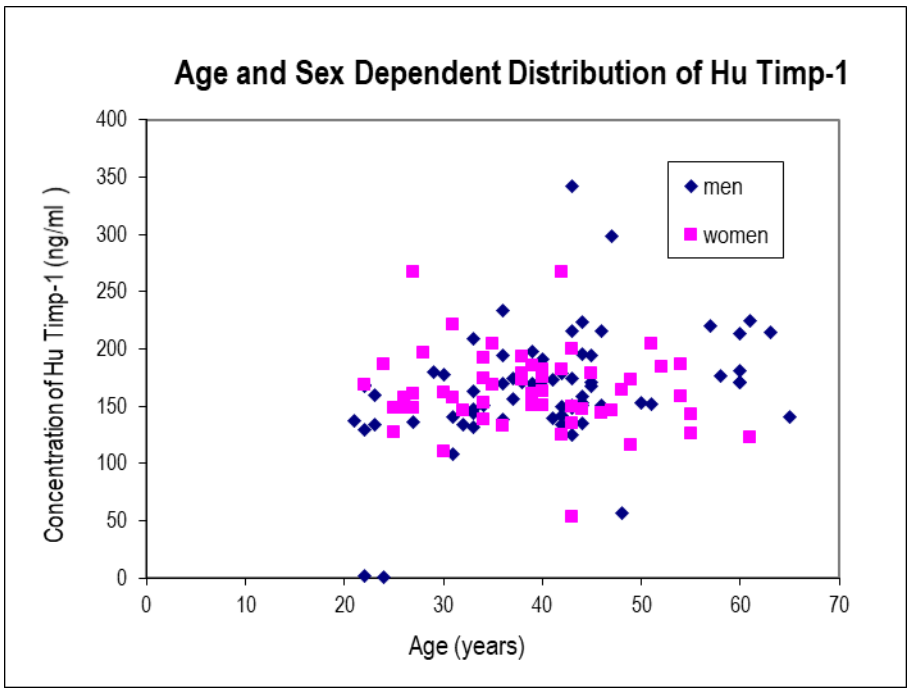


Figure 4: Human Timp-1 concentration plotted against donor age and sex.

- **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 references ranges for TIMP-1 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human TIMP-1 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 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 and samples

18. REFERENCES

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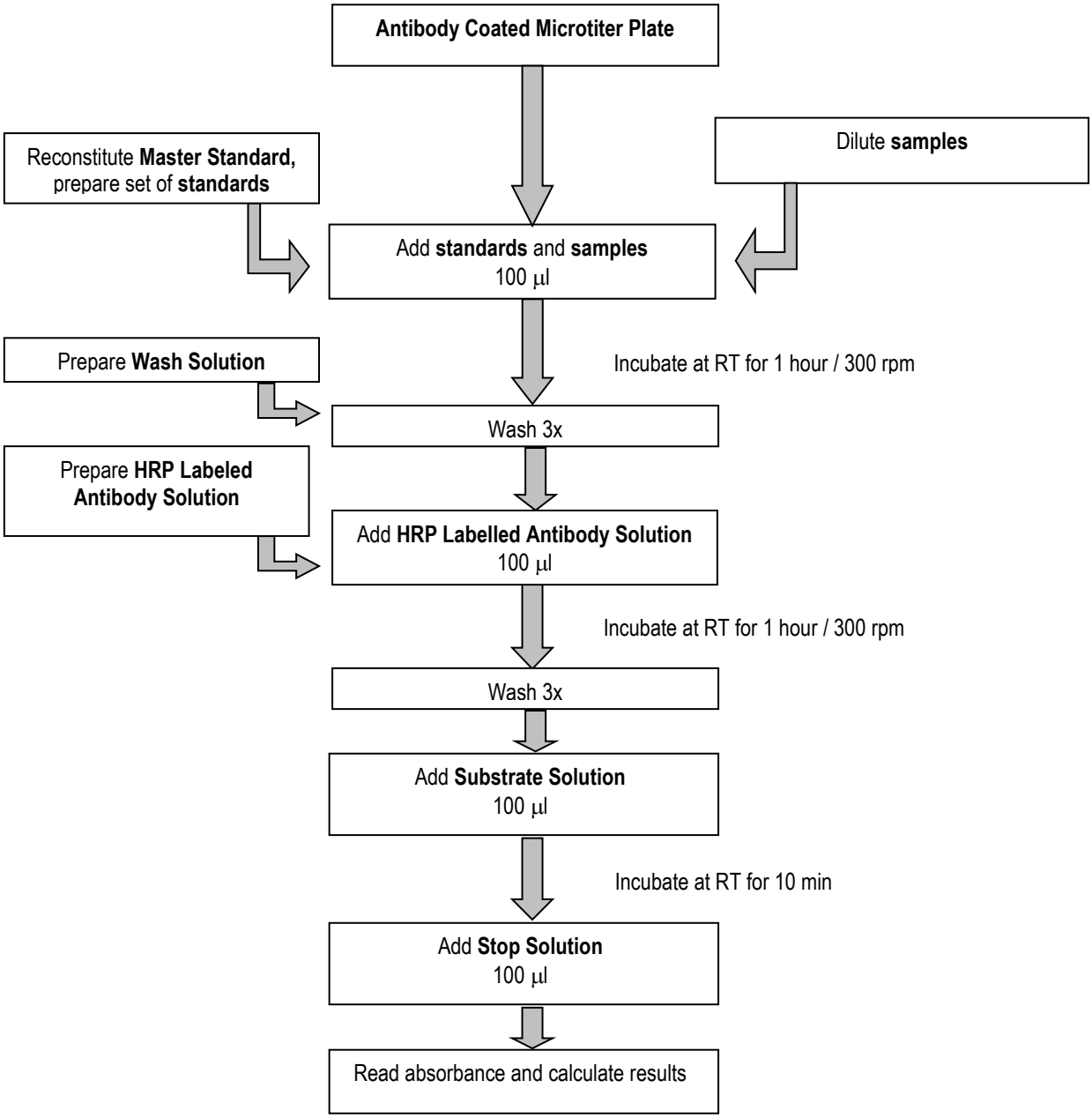
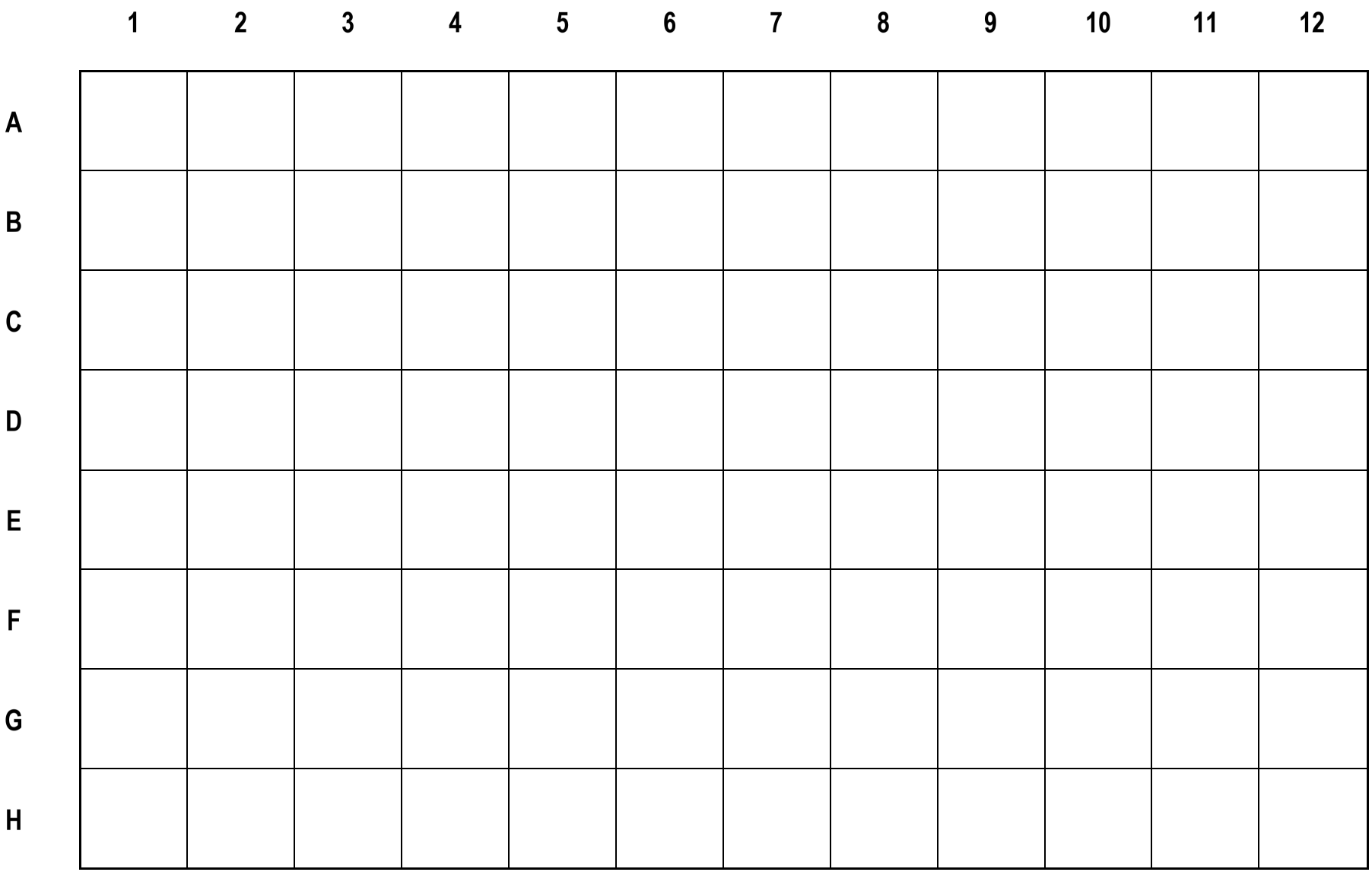
For more references on this product see our WebPages at www.biovendor.com

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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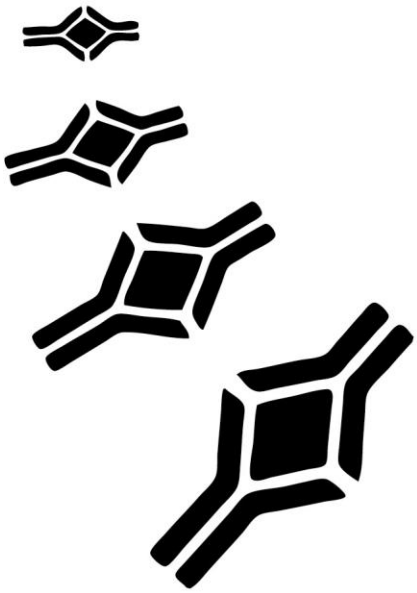

BioVendor – Laboratorní medicína a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

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