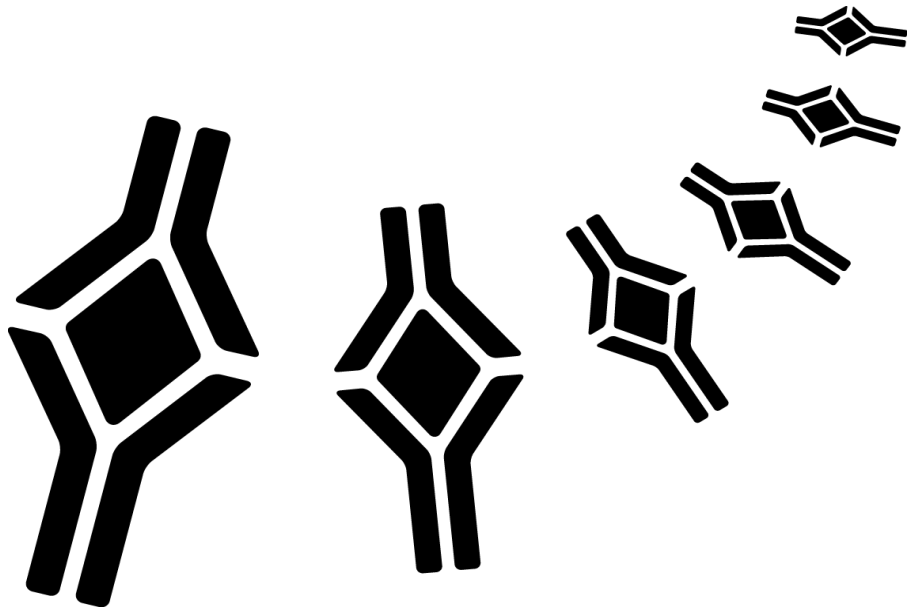
BioVendor
Research
and Diagnostic Products



HUMAN CTRP1 ELISA

Product Data Sheet

Cat. No.: RD191153100R

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 Human CTRP1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human CTRP1 protein.

Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures CTRP1 in serum and plasma citrate
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein
- 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

CTRP1 (Complement C1q tumor necrosis factor-related protein 1) is a member of the CTRP superfamily and is localized to human chromosome 17. It is a 32 kDa secreted glycoprotein comprised of 281 amino acids encoded by the human gene C1QTN1.
This protein is a highly conserved paralogue of adiponectin, containing a cluster of collagen-like repeats, a C-terminal globular 'C1q-like' domain and an N-terminal signal peptide sequence followed by a variable region and hence is predicted to be secreted protein.
CTRP1 is primarily expressed from cells in the stroma vascular fraction of adipose tissue and is also specifically expressed in the zona glomerulosa of the adrenal cortex and in vascular wall tissue. This protein is ubiquitous in most regions of the human brain and is particularly abundant in the spinal cord, but the pattern of expression and functions of CTRP1 in brain are completely unknown. Expression of CTRP1 (like adiponectin) is induced by PPARγ (peroxisome-proliferator-activated receptor γ). The LPS -induced increase in CTRP1 gene expression is found to be mediated by TNF-alpha and IL-1beta. Sequence homology inferred that the CTRP1 cytosolic domain is folded as a collagen-like helix followed by a globular domain. The interaction of the globular domain with the V2R was confirmed by pull-down experiments indicating that this structural motif can also interact with cytosolic proteins.
Obesity and the metabolic syndrome are frequently associated with elevated levels of aldosterone and the level of CTRP1 is increased in the blood of hypertensive patients, as aldosterone production was stimulated by CTRP1. It is well known that obesity is the leading cause of hypertension. CTRP1 is stimulating aldosterone production through induction of CYP11B2 gene expression. Therefore, CTRP1 could be a molecular link between obesity and hypertension.

<u>Areas of investigation:</u>
Energy metabolism and body weight regulation
Metabolic syndrome

4. TEST PRINCIPLE

In the BioVendor Human CTRP1 ELISA, the standards, quality controls and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human CTRP1 antibody. After 60 minutes incubation and washing, polyclonal anti-human CTRP1 antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured CTRP1. 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 CTRP1. 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. 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	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	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 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 550-650nm)
- 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 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 CTRP1 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 CTRP1 in the stock solution is **100 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	100 ng/ml
300 µl of stock	300 µl	50 ng/ml
300 µl of 50 ng/ml	300 µl	25 ng/ml
300 µl of 25 ng/ml	300 µl	12.5 ng/ml
300 µl of 12.5 ng/ml	300 µl	6.25 ng/ml
300 µl of 6.25 ng/ml	300 µl	3.13 ng/ml

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

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).
The reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
The reconstituted Quality Controls must be used immediately.
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 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 CTRP1 in serum and plasma citrate.

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 serum or plasma samples 20x with Dilution Buffer just prior to the assay, e.g. 8 µl of sample + 152 µl of Dilution Buffer when assaying samples as singlets or preferably 15 µl of sample + 285 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°. 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 CTRP1.

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 Standards, reconstituted Quality Controls 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. 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. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
8. Incubate the plate for **15 minutes** at room temperature. (The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C). No shaking!
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 CTRP1 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 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 100**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 50**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 25**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 12,5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 6,25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 3,13**	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 microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the 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 CTRP1 (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay; e.g. 10 ng/ml (from standard curve) x 20 (dilution factor) = 200 ng/ml.

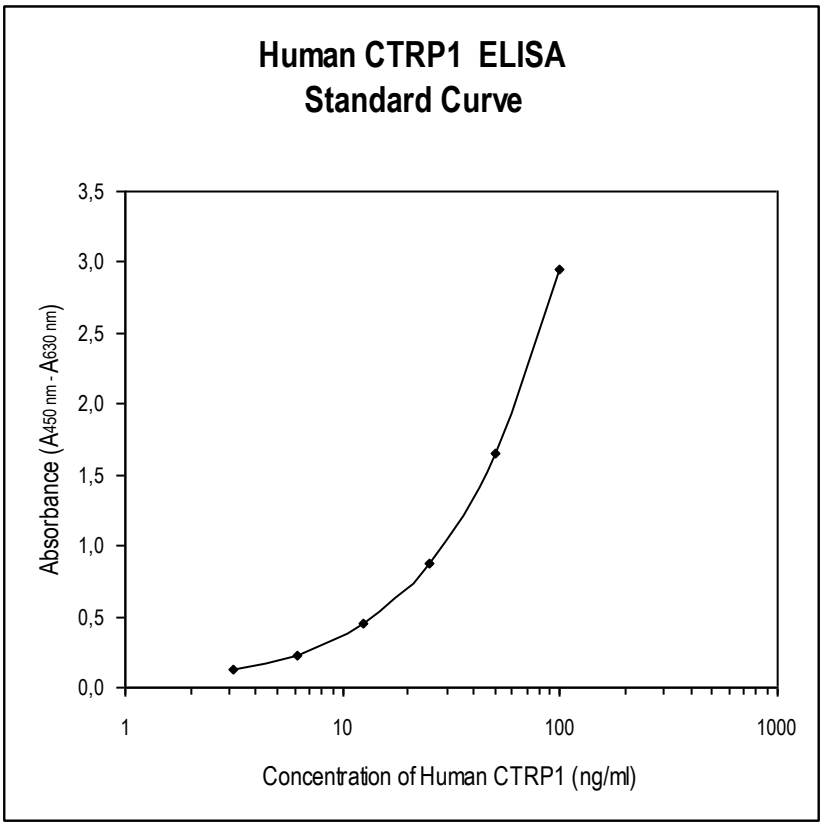


Figure 2: Typical Standard Curve for Human CTRP1 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human CTRP1 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 CTRP1 values in wells and is 0.016 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding CTRP1 level of 100 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the CTRP1 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human CTRP1.

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	yes
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	148.9	3.8	2.6
2	449.0	12.2	2.7

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	98.9	9.0	9.1
2	416.8	32.8	7.9

- **Spiking Recovery**

Serum samples were spiked with different amounts of human CTRP1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	260.4	-	-
	353.5	350.4	100.9
	411.4	440.4	93.4
	663.5	620.4	106.9
2	250.5		-
	312.3	340.5	91.7
	365.9	430.5	85.0
	559.3	610.5	91.6

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	955.5	-	-
	2x	473.2	477.8	99.1
	4x	236.8	238.9	99.1
	8x	120.3	119.4	100.7
2	-	1649.6	-	-
	2x	794.0	824.8	96.3
	4x	401.2	412.4	97.3
	8x	224.4	206.2	108.8

- **Effect of sample matrix**

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

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		Heparin	*Citrate*	*EDTA*
1	234.5	346.4	303.0	305.1
2	291.2	247.9	363.9	360.0
3	169.5	189.1	221.9	251.8
4	288.8	327.8	419.7	370.1
5	205.4	228.5	258.0	336.7
6	166.5	156.0	198.5	305.4
7	151.9	267.8	247.9	257.7
8	199.3	178.7	213.8	301.3
9	165.8	128.4	238.1	258.6
10	173.8	135.0	199.4	218.0
Mean (*n*g/ml)	**204.7**	**220.6**	**266.4**	**296.5**
Mean Plasma/Serum (%)		**107.8**	**130.2**	**144.8**
Coefficient of determination R^2		**0.41**	**0.83**	**0.70**

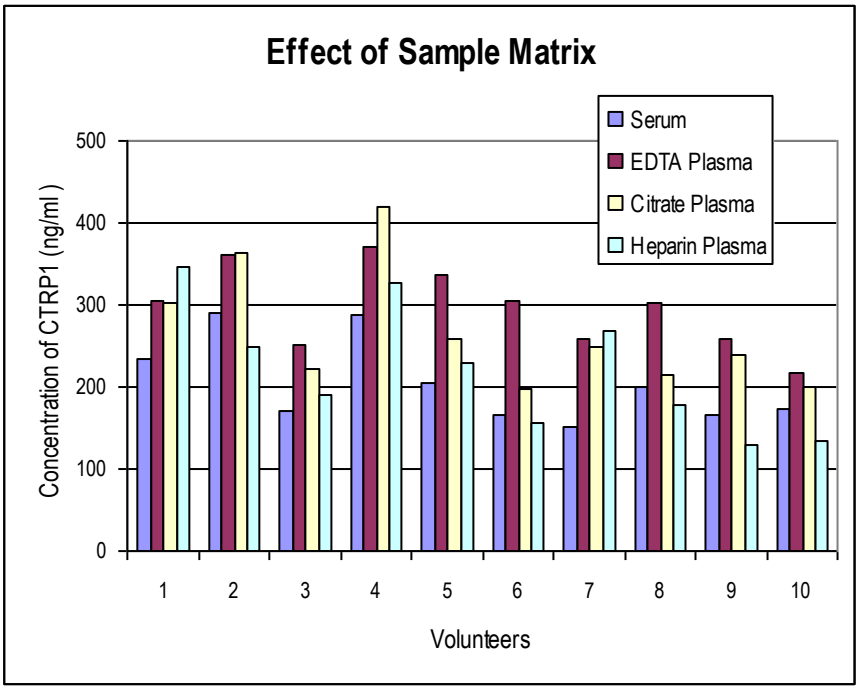


Figure 3: CTRP1 levels measured using Human CTRP1 ELISA from 10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

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

Samples should be stored at –20°C. However, no decline in concentration of CTRP1 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 0.05%, respectively.

Sample	*Incubation Temp, Period*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			Heparin	*Citrate*	*EDTA*
1	-20°C	778.6	606.4	810.9	810.0
	2-8°C, 1 day	887.5	643.5	870.2	1021.6
	2-8°C, 7 days	810.4	665.7	883.8	887.0
2	-20°C	627.3	740.1	772.9	1011.3
	2-8°C, 1 day	762.2	701.6	809.9	842.6
	2-8°C, 7 days	701.1	639.7	717.5	789.4
3	-20°C	948.2	919.7	1142.1	1278.9
	2-8°C, 1 day	945.4	877.2	987.9	1406.3
	2-8°C, 7 days	789.9	634.5	891.7	1132.2

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human CTRP1 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)*		
			Heparin	*Citrate*	*EDTA*
1	1x	673.8	553.5	932.8	1115.0
	3x	713.6	504.8	955.9	1047.5
	5x	660.8	563.0	1285.2	744.9
2	1x	734.1	592.3	891.4	1007.6
	3x	700.6	590.7	996.0	1028.6
	5x	612.6	632.4	817.1	757.9
3	1x	750.4	1132.7	1155.6	1433.3
	3x	773.6	1093.7	1262.9	1527.8
	5x	785.6	801.9	912.9	1439.5

14. DEFINITION OF THE STANDARD

The recombinant human CTRP1 is used as the Standard. The recombinant human CTRP1, produced in *E. coli*, is 30.45 kDa protein containing 266 amino acid residues of the human CTRP1 and 10 additional amino acid residues- His Tag.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum from 121 unselected donors (50 women + 71 men), 18-84 years old were assayed with Biovendor Human CTRP1 ELISA kit in our laboratory.
The presented data should be regarded only as guideline.

- **Age and sex dependent distribution of CTRP1**

Sex	*Age years*	*n*	*Mean*	*SD*	*Min.*	*Max.*	*Median*
			CTRP1 (ng/ml)				
Men	18-49	62	416.4	413.0	87.4	2096.9	252.6
	50-94	9	537.5	360.1	148.8	1 172.2	312.2
Women	18-48	44	246.9	124.9	86.9	889.6	227.2
	53-84	6	334.4	313.6	132.2	1 026.9	215.7

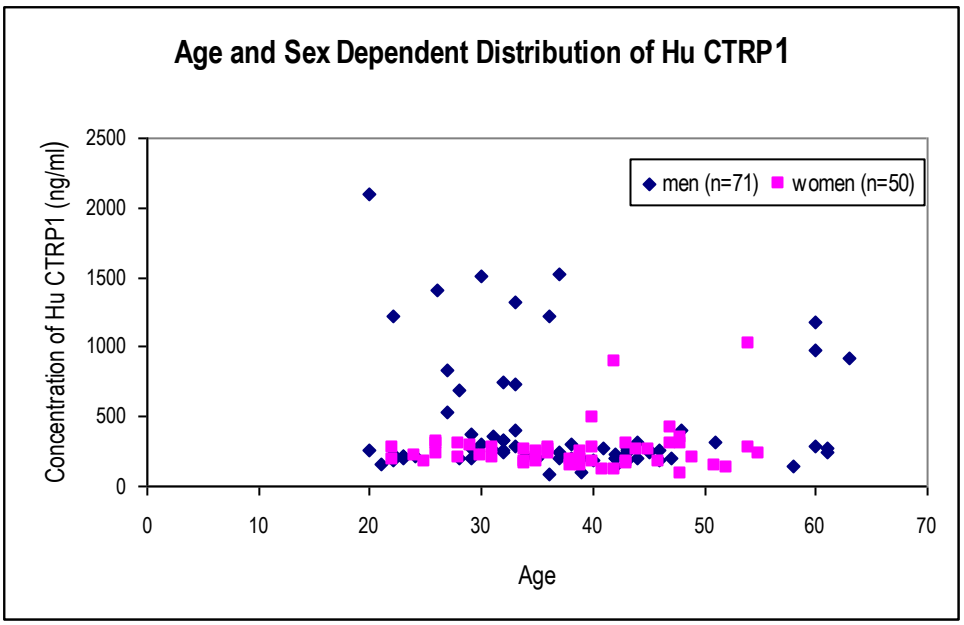


Figure 4: CTRP1 concentration plotted against donor age.

- **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 CTRP1 levels with the assay.

16. METHOD COMPARISON

The Biovendor Human CTRP1 ELISA was not compared to the other commercial immunoassays.

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

18. REFERENCES

References to CTRP1:

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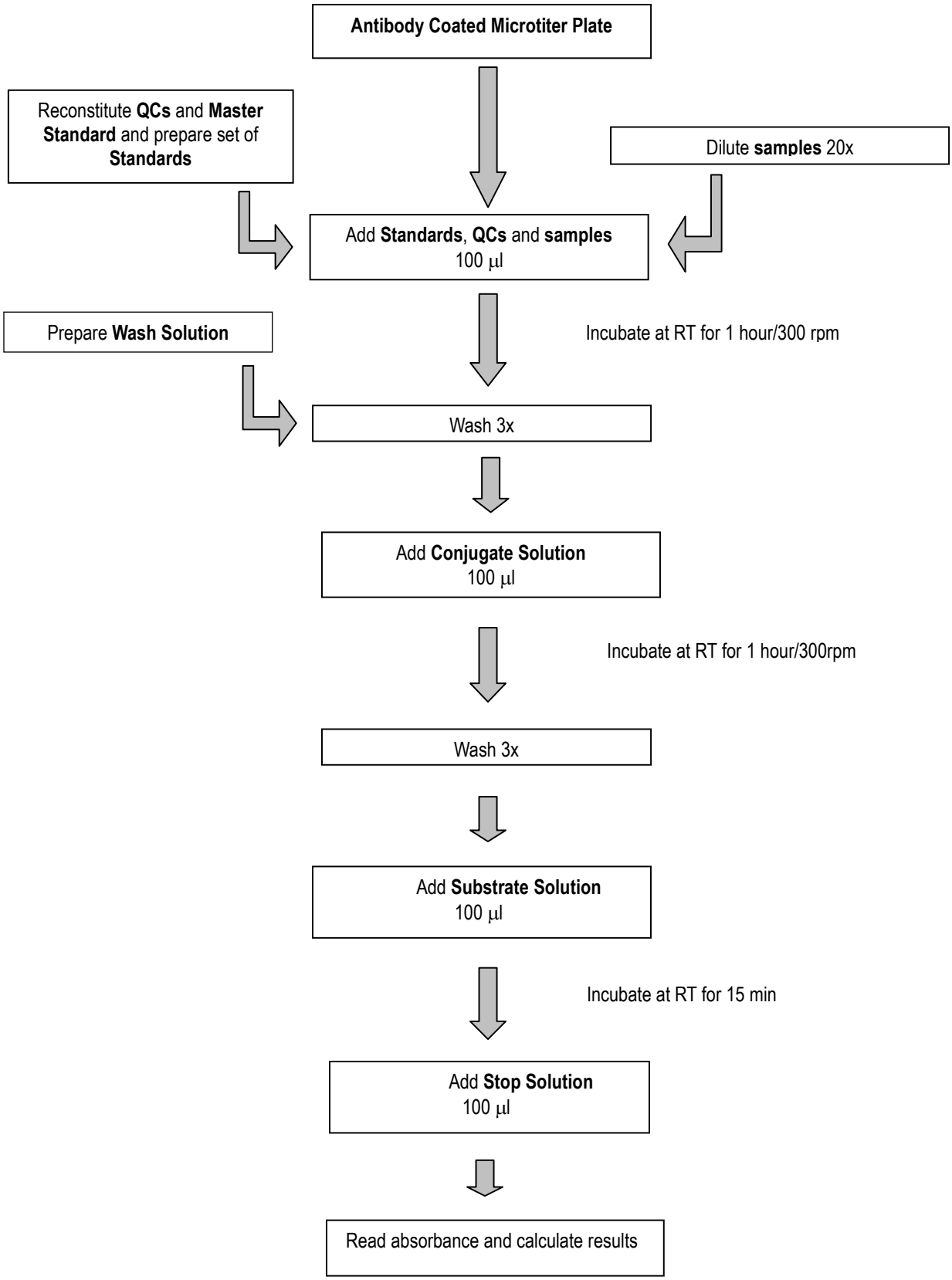
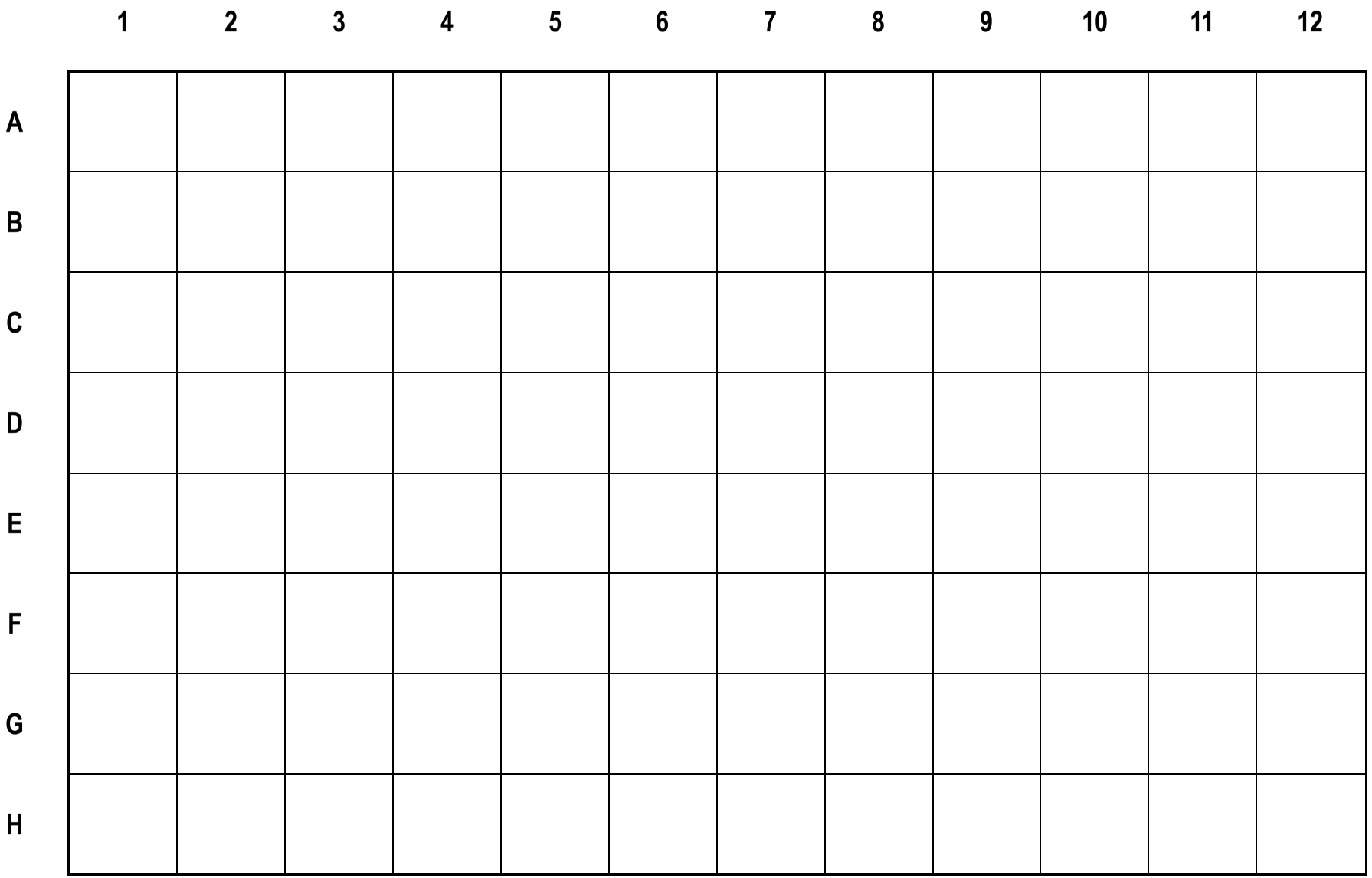
For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	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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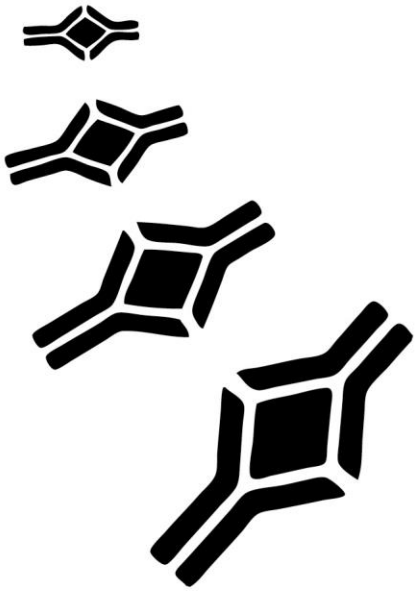

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