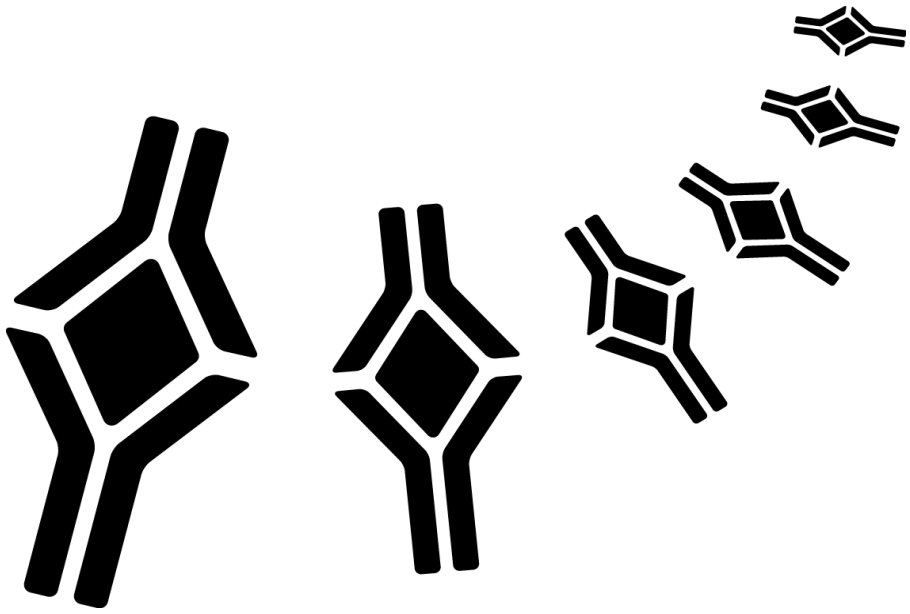
BioVendor
Research
and Diagnostic Products



HUMAN COLLAGEN TRIPLE HELIX REPEAT-CONTAINING 1 PROTEIN ELISA

Product Data Sheet

Cat. No.: RD191394200R

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 RD191394200R Human CTHRC1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human collagen triple helix repeat-containing protein 1 (CTHRC1).

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures human CTHRC1 in serum, plasma (EDTA, citrate, heparin) and tissue extracts
- 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

Collagen triple helix repeat-containing protein 1 (CTHRC1) was identified in a screen for differentially expressed sequences in balloon-injured versus normal arteries. Cthrc1 expression was not detectable in normal arteries. However, on injury it was transiently expressed by fibroblasts of the remodeling adventitia and by smooth muscle cells of the neointima. It was also found in the matrix of calcifying human atherosclerotic plaques. CTHRC1 is a secreted 28-kDa protein that is glycosylated and highly conserved from lower chordates to mammals. A short collagen motif with 12 Gly-X-Y repeats appears to be responsible for trimerization of the protein and this renders the molecule susceptible to cleavage by collagenase [1].

CTHRC1 is highly associated with calcified tissues and cartilaginous matrix, but not with endothelial cells. This is consistent with expression study results that showed CTHRC1 to be highly expressed in the developing skull bones, ribs, vertebrae, and cartilage primordia [2].

CTHRC1 is a circulating factor detectable qualitatively in plasma of healthy human subjects. [3]. CTHRC1 plasma levels were also significantly elevated during pregnancy, in diabetes, in inflammatory and infectious conditions, in subjects with acute myeloid leukemia but not in subjects with solid cancers [4]. Hormonal functions of CTHRC1 include regulation of lipid storage and cellular glycogen levels with potentially broad implications for cell metabolism and physiology [3]. Deletion of the CTHRC1 gene leads to fatty liver (steatosis) formation in mice [3] while others showed that inactivation of this gene also results in low bone mass [5]. CTHRC1 was identified in skeletal and cardiac muscles of mice, representing Duchenne and congenital muscle dystrophies (DMD and CMD) and dysferlinopathy [6]. CTHRC1 is involved in postnatal bone formation [5] and is secreted by mature functional osteoclasts. It acts on stromal/osteoblastic cells by binding to a putative cell surface receptor in order to stimulate osteoblastic differentiation as well as recruitment, thereby promoting bone formation [7].

Among the cancer cells reported to express CTHRC1 are melanomas [8, 9], hepatocellular carcinoma [10], oral cancer [13], breast ductal carcinoma [12,13], pancreatic cancer [14], colorectal cancer [15,16], gastric cancer [17], and dermatofibrosarcoma protuberans [18], but a more recent study demonstrated that CTHRC1 expression in human cancers originates from activated stromal cells, not from the tumor cells themselves. [4].

CTHRC1 may have therapeutic value in antifibrotic treatment strategies [1] and could have major clinical applications in the fields of vascular disease, repair, and fibrosis. [19]. The results indicate that CTHRC1 increases bone mass as a positive regulator of osteoblastic bone formation and offers an anabolic approach for the treatment of osteoporosis [6]. CTHRC1 could serve as a potential marker for predicting progression and metastasis of the aforementioned types of cancers.

<u>Areas of investigation:</u>

Cardiovascular disease
Coronary artery disease
Atherosclerosis
Bone and cartilage metabolism
Oncology
Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the BioVendor Human CTHRC1 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human CTHRC1 antibody. After 60 minutes incubation and a washing, biotin-labelled polyclonal anti-human CTHRC1 antibody is added and incubated with captured CTHRC1 for 60 minutes. 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 CTHRC1. A standard curve is constructed by plotting absorbance values against concentrations of CTHRC1 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 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	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
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 10 -1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

» **All reagents need to be brought to room temperature prior to use**

» **Always prepare only the appropriate quantity of reagents for your test**

» **Do not use components after the expiration date marked on their label**

- **Assay reagents supplied ready to use:**

Antibody Coated Microtiter Strips
Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with dessicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate
Biotin-Ab 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 CTHRC1 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 CTHRC1 in the stock solution is **2500 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	–	2500 pg/ml
250 µl of stock	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.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute Biotin Labelled Antibody Concentrate 100x with Biotin-Ab Diluent (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Biotin-Ab Diluent for 8 wells).
Stability and storage:
Do not store the reconstituted and/or diluted Biotin Labelled Antibody solutions.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution, e.g. 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use in 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 human CTHRC1 in serum, plasma (EDTA, citrate, heparin) and tissue extracts.

Samples can be assayed immediately after collection or should be stored at -20°C. Mix thawed samples thoroughly 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 and plasma is 10x.
Dilute samples (serum, plasma) **10x** with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets, or preferably 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Recommended starting dilution for tissue extracts is 20x.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C or lower for long-term storage. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

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 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 a 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 a 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 a 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 less 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 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 CTHRC1 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 two 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 2500**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 1250**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 625**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 313**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 156**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 78**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
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 CTHRC1 (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. 270 pg/ml (from standard curve) x 10 (dilution factor) = 2700 pg/ml = 2.7 ng/ml.

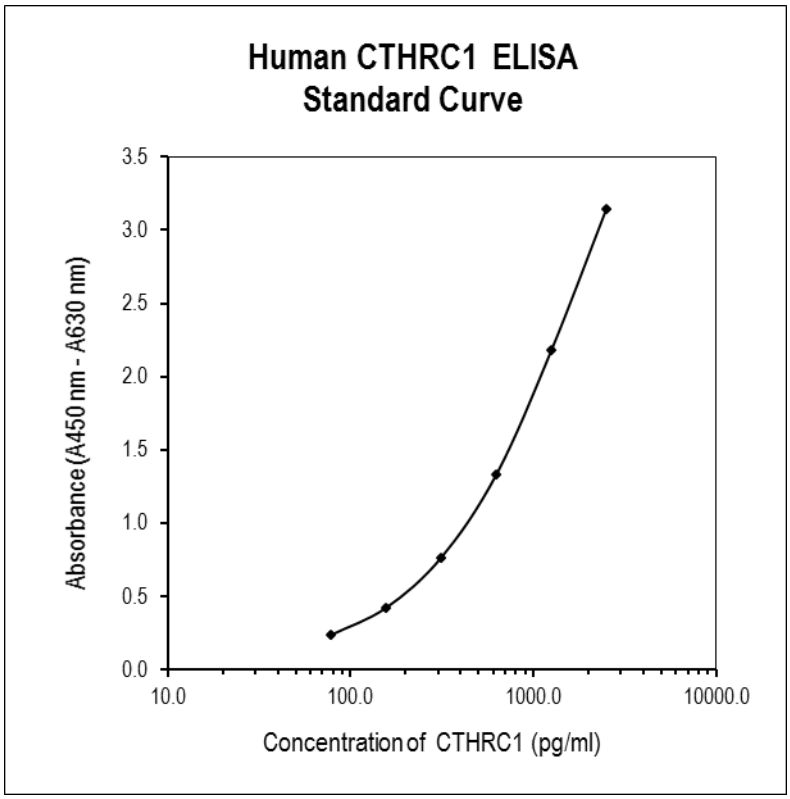


Figure 2: Typical Standard Curve for Human CTHRC1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human CTHRC1 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 CTHRC1 values in wells and is 27 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.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.90	0.18	6.3
2	5.19	0.28	5.5

Inter-assay (Run-to-Run) (n=8)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	3.70	0.17	4.5
2	5.08	0.29	5.7

- **Spiking Recovery**

Serum samples were spiked with different amounts of human CTHRC1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	1.32 2.11 2.71 4.30	- 2.10 2.88 4.44	- 100.6 94.1 96.7
2	1.23 2.02 2.88 4.24	- 2.01 2.79 4.35	- 100.4 100.3 97.4

- **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	- 2x 4x 8x	3.51 1.81 0.89 0.42	- 1.75 0.88 0.44	- 103.1 101.0 96.3
2	- 2x 4x 8x	4.25 2.11 0.95 0.54	- 2.13 1.06 0.53	- 99.3 88.9 101.8

- **Effect of sample matrix**

Citrate, heparin 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)*		
		EDTA	*Citrate*	*Heparin*
1	3.03	2.61	2.43	2.50
2	3.10	2.59	2.81	2.92
3	3.17	2.51	2.48	2.59
4	3.00	2.67	2.42	2.56
5	3.05	2.87	2.60	2.77
6	1.98	1.83	1.58	1.76
7	2.79	2.81	2.47	2.84
8	2.00	2.05	1.79	1.81
9	1.35	1.20	1.08	1.16
10	2.19	2.15	1.87	1.91
Mean (ng/ml)	**2.57**	**2.33**	**2.15**	**2.28**
Mean Plasma/Serum (%)		**90.8**	**83.8**	**88.8**
Coefficient of determination R^2		**0.87**	**0.95**	**0.91**

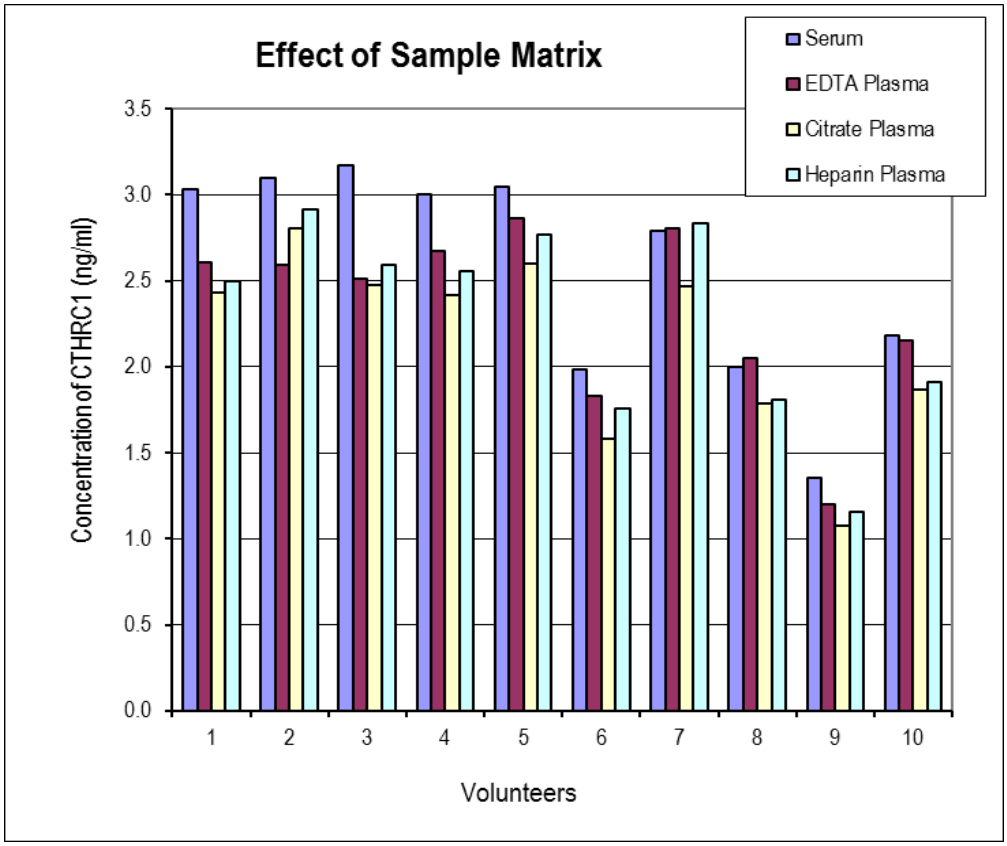


Figure 3: CTHRC1 levels measured using Human CTHRC 1 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein. The recombinant human CTHRC1, produced in HEK293 cell line, is 23.9 kDa protein consisting of 213 amino acid residues of human CTHRC1 (Ser31-Lys243) and 6 additional amino acids.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 154 unselected donors (89 men + 65 women) 20–65 years old were assayed with the BioVendor Human CTHRC1 ELISA in our laboratory.

Sex	*Age (years)*	*n*	*CTHRC1 (ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	20-29	18	2.51	2.38	0.66	1.68	3.88
	30-39	26	2.59	2.47	0.73	1.52	4.99
	40-49	31	2.67	2.56	0.54	1.81	3.98
	50-65	14	2.85	2.67	0.54	1.94	3.77
Women	20-29	12	1.63	1.56	0.40	1.07	2.49
	30-39	26	1.91	2.00	0.39	1.15	2.95
	40-49	20	2.24	2.29	0.51	1.44	3.41
	50-61	8	2.35	2.37	0.56	1.11	3.13

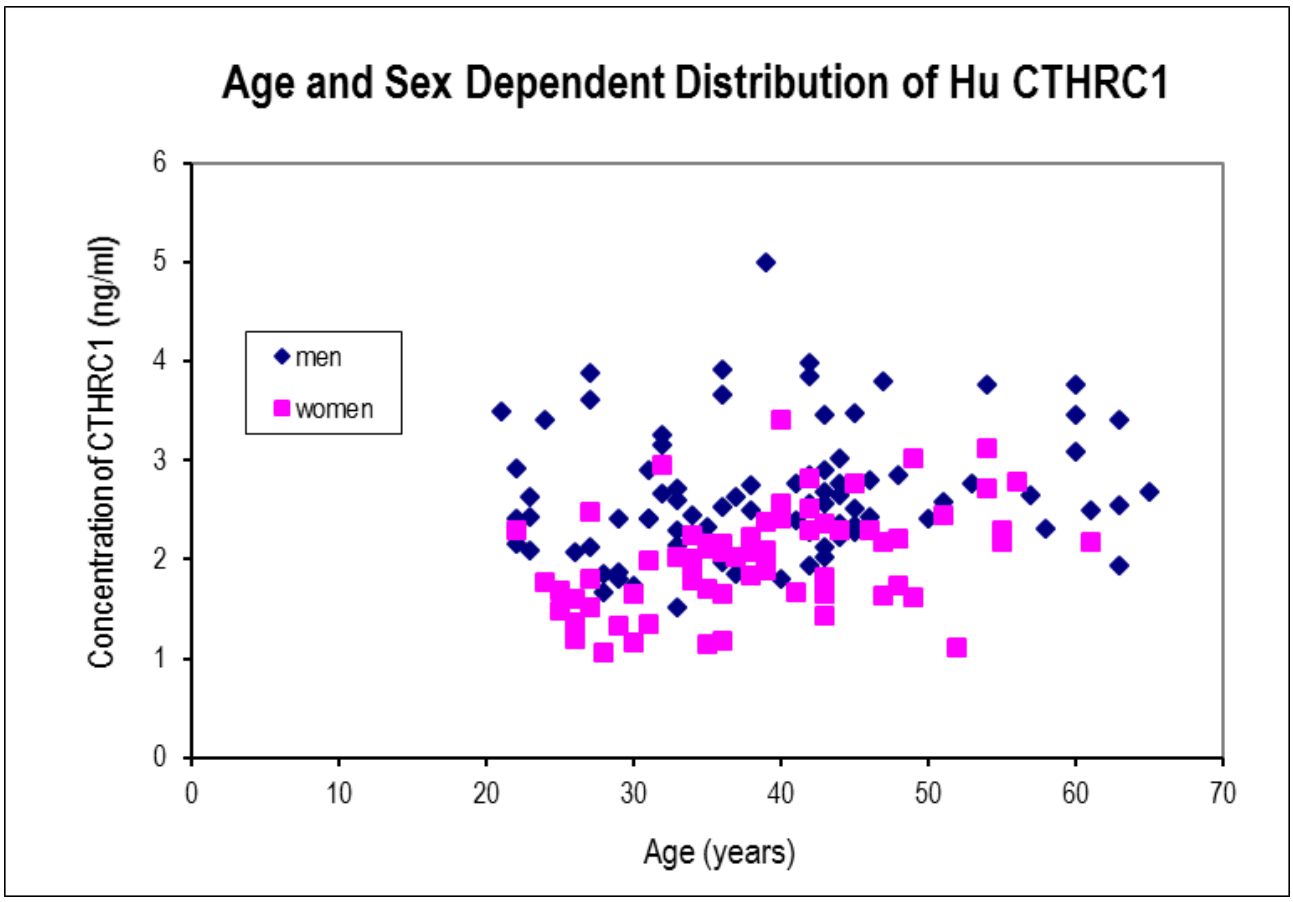


Figure 4: Human CTHRC1 concentration plotted against donor age and sex.

- **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 CTHRC1 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human CTHRC1 ELISA has not been compared to any 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
- 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

18. REFERENCES

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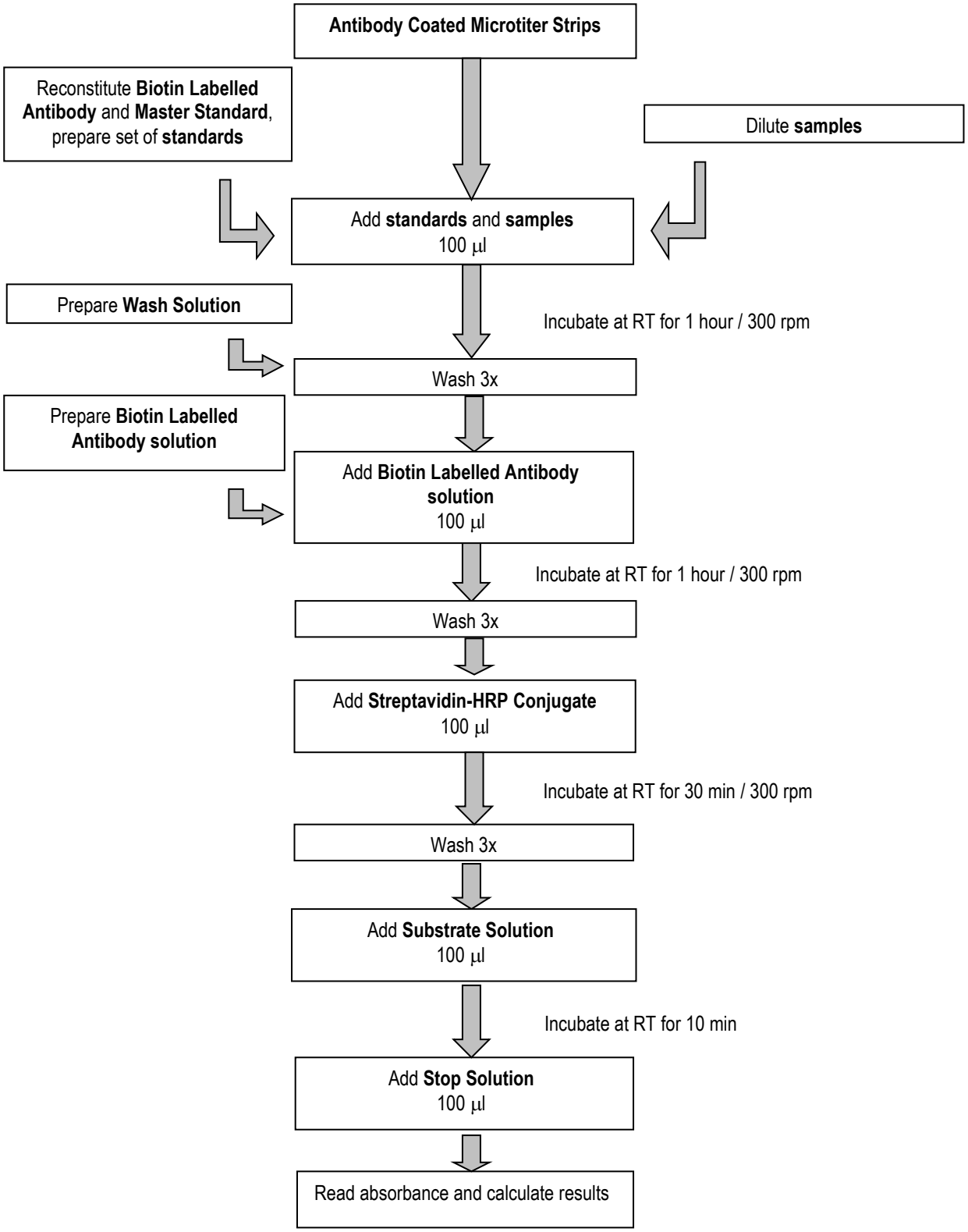
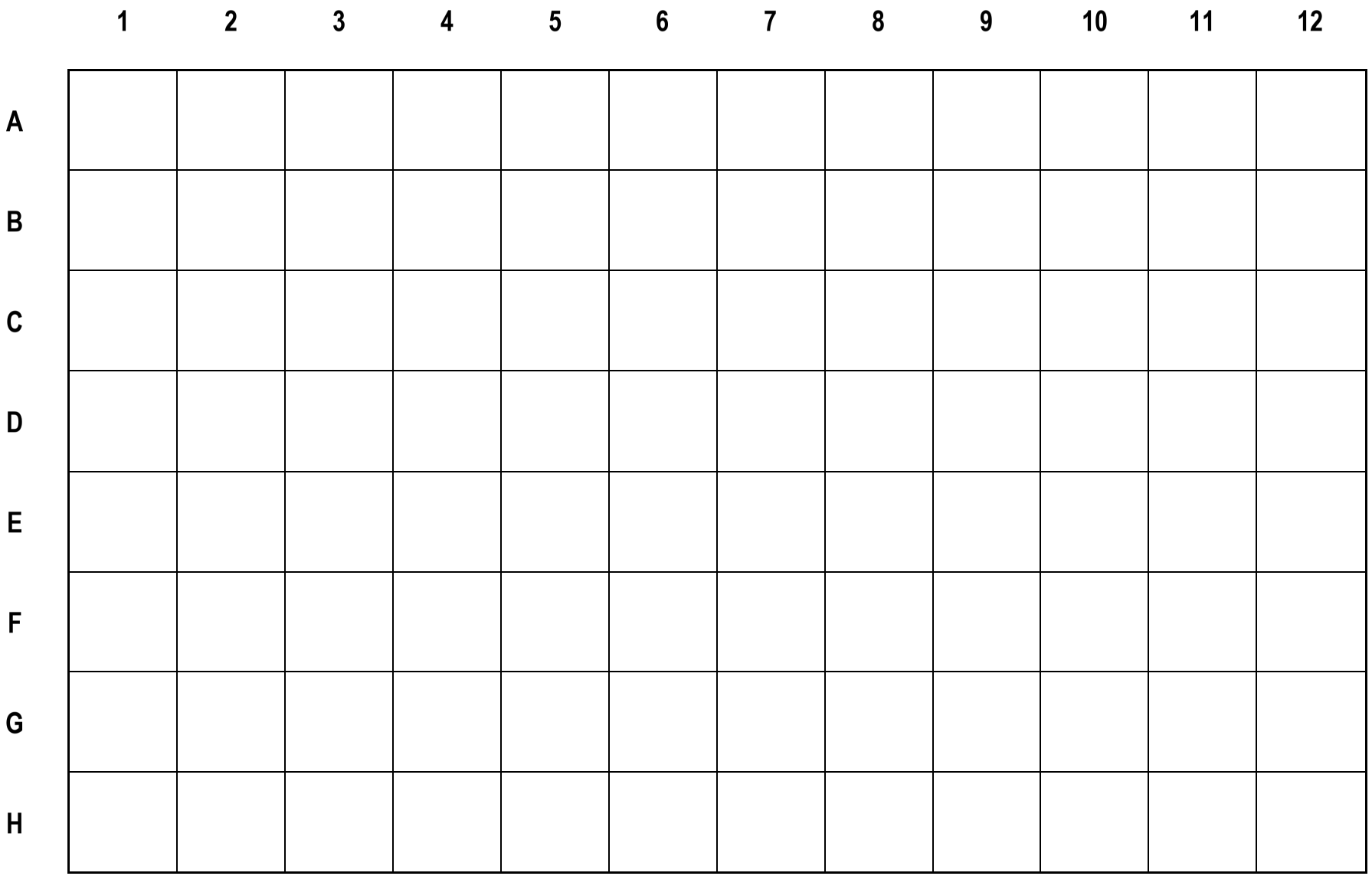
» For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	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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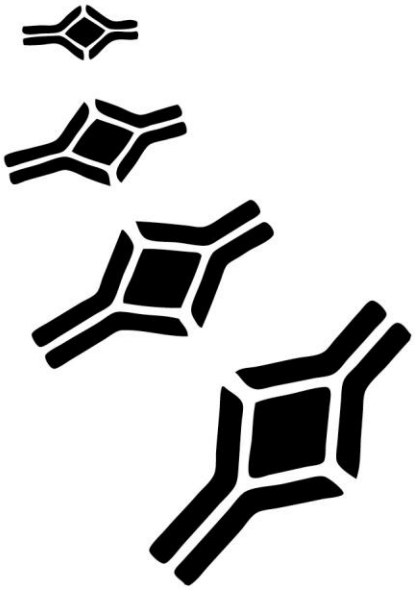

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