

ENG

Instructions for Use:
**HUMAN CONNECTIVE TISSUE
GROWTH FACTOR ELISA**

Catalogue number:
RD191035200R

For research use only.

 **BioVendor**
R&D[®]



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HISTORY OF CHANGES

Previous version	Current version
ENG.003.A	ENG.004.A
History of changes added.	
A symbol indicating the manufacturer added.	

1. INTENDED USE

The RD191035200R Human Connective Tissue Growth Factor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human connective tissue growth factor (CTGF).

Features

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

Connective Tissue Growth Factor (CTGF, CCN2, HCS24, IGFBP8, NOV2) is a heparin-binding glycoprotein with molecular weight of 36-38 KDa, composed of 343-349 amino acid residues and belonging to the CCN family. The CCN family of proteins is a complex family of multifunctional proteins containing six members designated CCN1 to CCN6 (1,2).

CTGF/CCN2 is a secreted protein with major roles in angiogenesis, chondrogenesis (cartilage regeneration), osteogenesis, tissue repair, cancer and fibrosis (cell adhesion, migration and proliferation). The primary function of CTGF is to modulate and coordinate signaling responses involving cell surface proteoglycans and growth factors. Abnormal amplification of CTGF dependent signals results in failure to terminate tissue repair, leading to pathological scarring in conditions such as fibrosis and cancer (3).

The role of CTGF in fibrogenesis suggests this protein as a potential fibrogenic marker, because CTGF expression is induced by transforming growth factor β (TGF- β), which is the most important fibrogenic cytokine, and also, CTGF is expressed increasingly in major profibrogenic cell types (hepatocytes, biliary epithelial cells and hepatic stellate cells) during their transdifferentiation in extracellular matrix-producing myofibroblasts. The expression of CTGF is strongly upregulated in fibrotic liver tissue and CTGF is secreted into extracellular space, thus, it can reach the systemic circulation directly. The mean concentration of CTGF was the highest in serum of patients with fibrosis and chronic viral hepatitis, but lower in patients with fully developed cirrhosis. The data

confirm hypothesis that CTGF increases in the circulation of patients with active, fibrogenic liver diseases (4,5,6). No statistical relations between CTGF levels and parameters of liver injury (AST, ALT) were noticed, but CTGF levels in serum are correlated negatively with serum albumin levels and platelet counts. CTGF was reported to be associated with and released by platelets during the coagulation process.

In hepatocellular carcinoma (HCC) patients, CTGF concentrations decreased with tumor progression and size (7). Increase in CTGF concentration in plasma, serum and urine have been suggested as a surrogate marker of fibrotic disease activity in scleroderma, pulmonary fibrosis, diabetic nephropathy (DN) and glomerulosclerosis (8,9,10).

CTGF appears to be an important growth factor implicated in the development of diabetes complications. Plasma CTGF-N (NH₂-terminal fragment) concentrations are elevated in type 1 diabetic patients with nephropathy and correlate with proteinuria and creatinine clearance (9). In the previous study it was found that the presence of CTGF in urine and the relationship to diabetes and renal disease in an experimental animal model (rats). Low levels of urinary CTGF were found in healthy rats, but higher levels were found in diabetic animals (10). In DN patients, urinary CTGF (uCTGF) was independently associated with markers of proximal and distal tubular dysfunction and damage. Urinary CTGF levels in nephropathy may indicate the patients who are destined for progressive glomerulosclerosis and end-stage renal disease (ESRD). In conclusion, uCTGF in DN patients is elevated as a result of both increased local production and reduced reabsorption due to tubular dysfunction (11). It was observed that serum CTGF levels were significantly higher in non-renal systemic lupus erythematosus and correlated with chronic renal interstitial injury and doubling of serum creatinine in patients with lupus nephritis (12).

CTGF was the most abundantly expressed growth factor present in chondrocytes of patients with osteoarthritis (OA). CTGF was detected in plasma and synovial fluid of patients with primary knee osteoarthritis and positively correlated with radiographic grading of knee OA (13).

Results of several studies suggest that myocardial CTGF/CCN2 is upregulated in heart failure of both ischemic and non-ischemic etiologies in experimental models as well as humans (14,15). Plasma CTGF levels have also been reported to be elevated in heart failure patients and correlated with NYHA-class (the New York Heart Association Functional Classification). This study indicates that CTGF concentration in plasma is a novel diagnostic marker for cardiac dysfunction and myocardial fibrosis in chronic heart failure patients (16). Increased myocardial CTGF activities after myocardial infarction are associated with attenuation of left ventricular (LV) remodeling and improved LV function mediated by attenuation of inflammatory responses and inhibition of apoptosis (14).

Plasma CTGF levels were elevated in patients with stable asthma and were correlated with parameters of pulmonary function tests and asthma control (17).

Areas of investigation:

Cardiovascular disease

Coronary artery disease

Renal disease

Pulmonary disease

Liver

Rheumatoid arthritis (RA)

Diabetology

Oncology

Apoptosis

4. TEST PRINCIPLE

In the BioVendor Human Connective Tissue Growth Factor ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human CTGF antibody. After 60 minutes incubation and washing, biotin-labelled polyclonal anti-human CTGF antibody is added and incubated with captured CTGF 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 CTGF. A standard curve is constructed by plotting absorbance values against concentrations of CTGF 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
Biotin Labelled Antibody	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	2 x 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 (Dilution Buffer)
- Precisie pipettes to deliver 10 -1000 μ l with disposable tips
- Multichannel pipette to deliver 100 μ l 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

Human CTGF 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 (do not foam).

The resulting concentration of CTGF in the stock solution is **20 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	–	20 ng/ml
250 µl of stock	250 µl	10 ng/ml
250 µl of 10 ng/ml	250 µl	5 ng/ml
250 µl of 5 ng/ml	250 µl	2.5 ng/ml
250 µl of 2.5 ng/ml	250 µl	1.25 ng/ml
250 µl of 1.25 ng/ml	250 µl	0.63 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.

Biotin Labelled Antibody

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Biotin Labelled Antibody!!!

Reconstitute the lyophilized Biotin Labelled Antibody with Dilution Buffer 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 Dilution Buffer (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Dilution Buffer 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 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 total human CTGF in serum, plasma (EDTA, citrate, heparin) and urine samples.

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

Limitation in using serum samples: It is recommended to use a careful technique to collect blood samples. Platelet derived CTGF (full-length CTGF) may be released into the serum by platelet activation during or after blood collection, thereby interfering with accurate determination of the serum CTGF level. Thus, serum CTGF levels are significantly higher than plasma levels. Therefore, rapid separation of blood samples from cellular components is essential for preserving true CTGF levels in samples for later analysis.

Please see Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human CTGF.

An appropriate dilution should be assessed by the researcher in advance to batch measurement.

Recommended starting dilution for plasma and serum is 5x. Dilute plasma or serum samples 5x with Dilution Buffer just prior to the assay, e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 50 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Recommended starting dilution for urine is 3x. Dilute urine samples 3x with Dilution Buffer just prior to the assay, e.g. 40 µl of sample + 80 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°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 diluted Standards, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against 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 5-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 5-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 30 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 CTGF concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 20	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	Standard 10	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	Standard 5	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	Standard 2.5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	Standard 1.25	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	Standard 0.63	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 CTGF (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 5 (dilution factor) = 50 ng/ml.

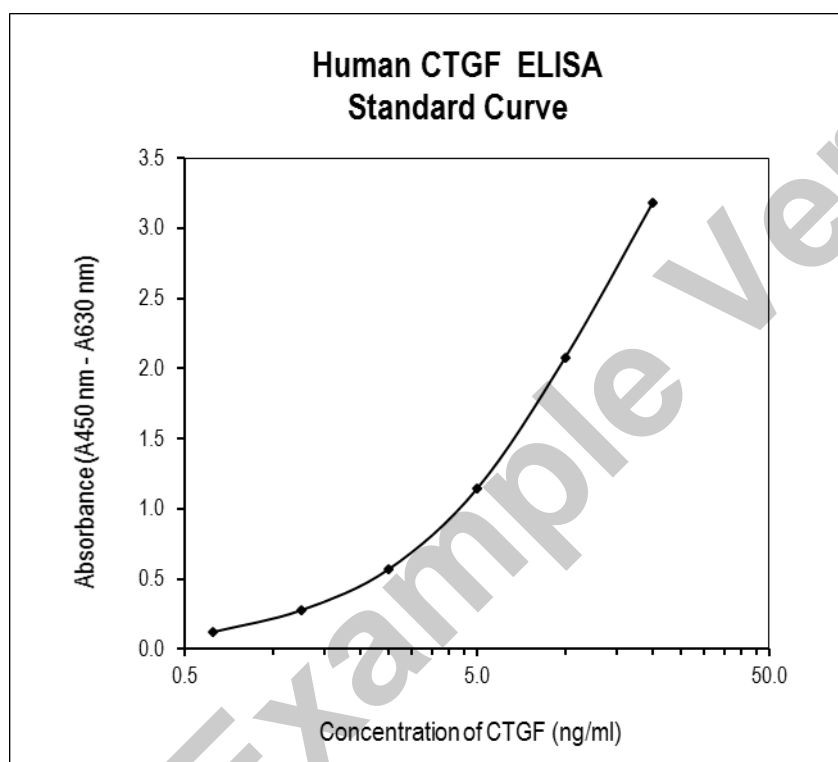


Figure 2: Typical Standard Curve for Human Connective Tissue Growth Factor ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Connective Tissue Growth Factor 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: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real CTGF values in wells and is 0.02 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.

Specificity

The antibodies used in this ELISA are specific for human connective tissue growth 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	21.2	0.62	2.9
2	36.6	1.05	2.9

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	22.1	0.91	4.1
2	39.5	1.79	4.5

Spiking Recovery

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

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	13.4	-	-
	20.2	19.7	102.6
	26.7	25.9	103.1
	37.8	38.4	98.5
2	20.3	-	-
	31.9	32.8	97.1
	43.0	45.3	94.9
	67.8	70.3	96.4

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	38.0	-	-
	2x	18.2	19.0	95.6
	4x	8.57	9.50	90.2
	8x	4.17	4.75	87.6
2	-	69.4	-	-
	2x	33.9	34.7	97.8
	4x	15.5	17.4	89.0
	8x	7.33	8.68	84.5

Effect of sample matrix

Citrate and heparin plasma and serum samples were compared to respective EDTA plasma samples from the same 10 individuals. We obtained very low correlation among serum and plasma samples. Results are shown below:

Volunteer No.	EDTA plasma (ng/ml)	Citrate plasma (ng/ml)	Heparin plasma (ng/ml)	Serum (ng/ml)
1	18.5	10.1	14.9	52.4
2	27.1	16.4	22.2	71.9
3	16.4	9.00	12.4	75.2
4	18.6	12.4	14.2	80.7
5	12.4	9.2	10.6	41.4
6	16.7	11.9	15.0	84.1
7	20.0	12.2	16.1	89.8
8	20.4	12.6	17.9	95.3
9	12.9	9.00	10.7	49.7
10	99.9	55.7	19.4	80.5
Mean (ng/ml)	26.3	15.9	15.4	72.1
Mean samples/EDTA plasma (%)	-	60.5 %	58.6 %	274.1 %
Coefficient of determination R²	-	1.00	0.95	0.06

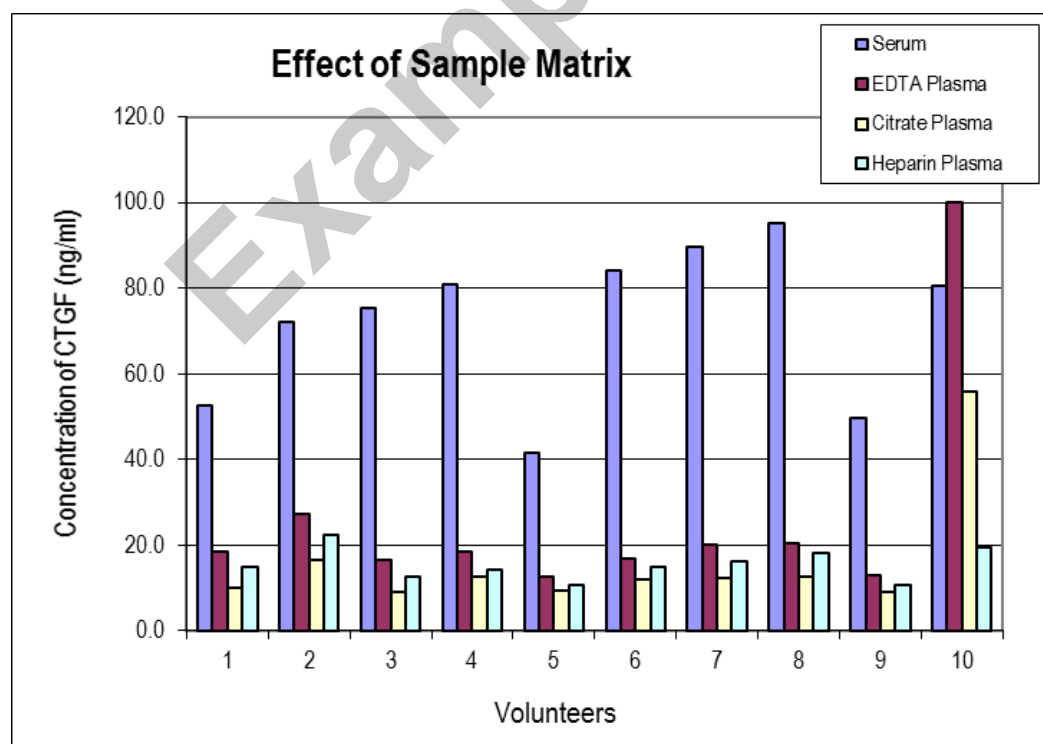


Figure 3: CTGF levels measured using Human Connective Tissue Growth Factor ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein. The recombinant human connective tissue growth factor, produced in HEK293, is 36 kDa protein consisting of 329 amino acid residues of human connective tissue growth factor and 6 additional amino acids.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20–65 years old were assayed with the BioVendor Human Connective Tissue Growth Factor ELISA in our laboratory.

Sex	Age (years)	n	CTGF (ng/ml)				
			Mean	Median	SD	Min	Max
Men	20-29	18	46.1	59.1	15.1	26.7	82.5
	30-39	26	45.7	44.6	14.0	22.7	93.3
	40-49	31	47.1	45.9	14.0	22.3	76.7
	50-65	14	43.2	44.9	6.83	29.8	51.9
Women	20-29	12	50.0	48.4	16.4	25.7	81.4
	30-39	26	43.3	40.9	12.7	19.7	82.2
	40-49	20	45.0	41.2	11.8	28.8	66.7
	50-61	8	43.4	40.4	9.8	31.0	66.1

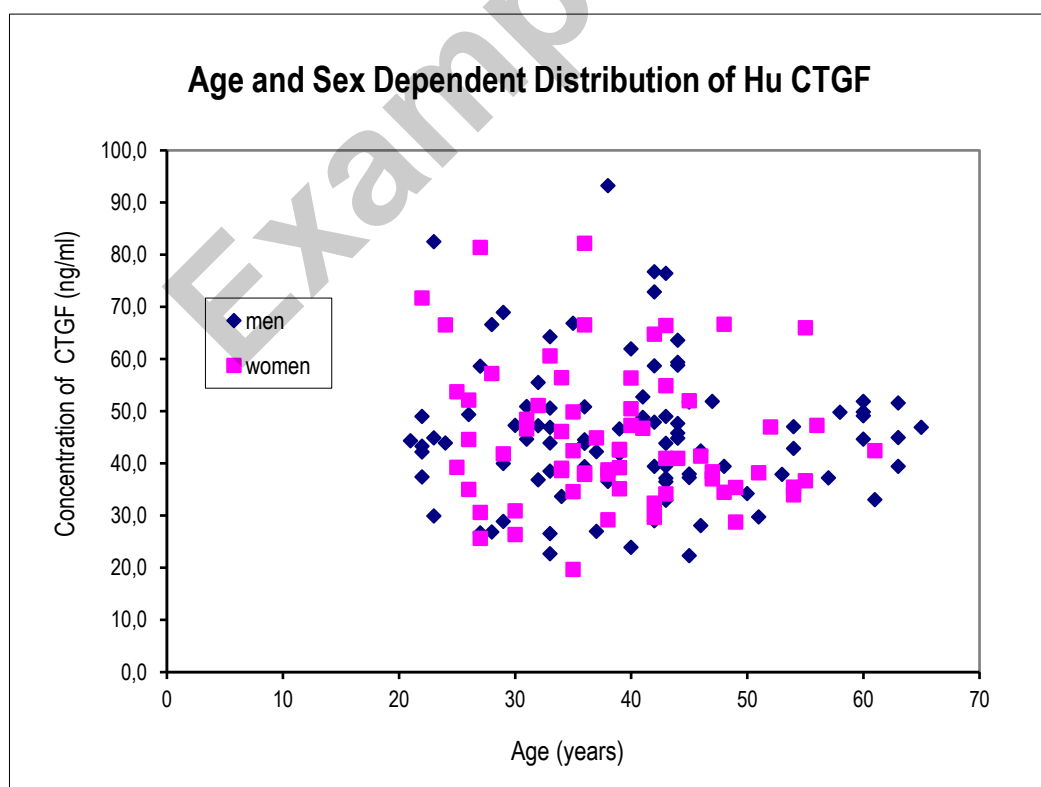


Figure 4: Human CTGF concentration plotted against donor age and sex.

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 sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for connective tissue growth factor levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Connective Tissue Growth Factor 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
- 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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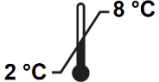




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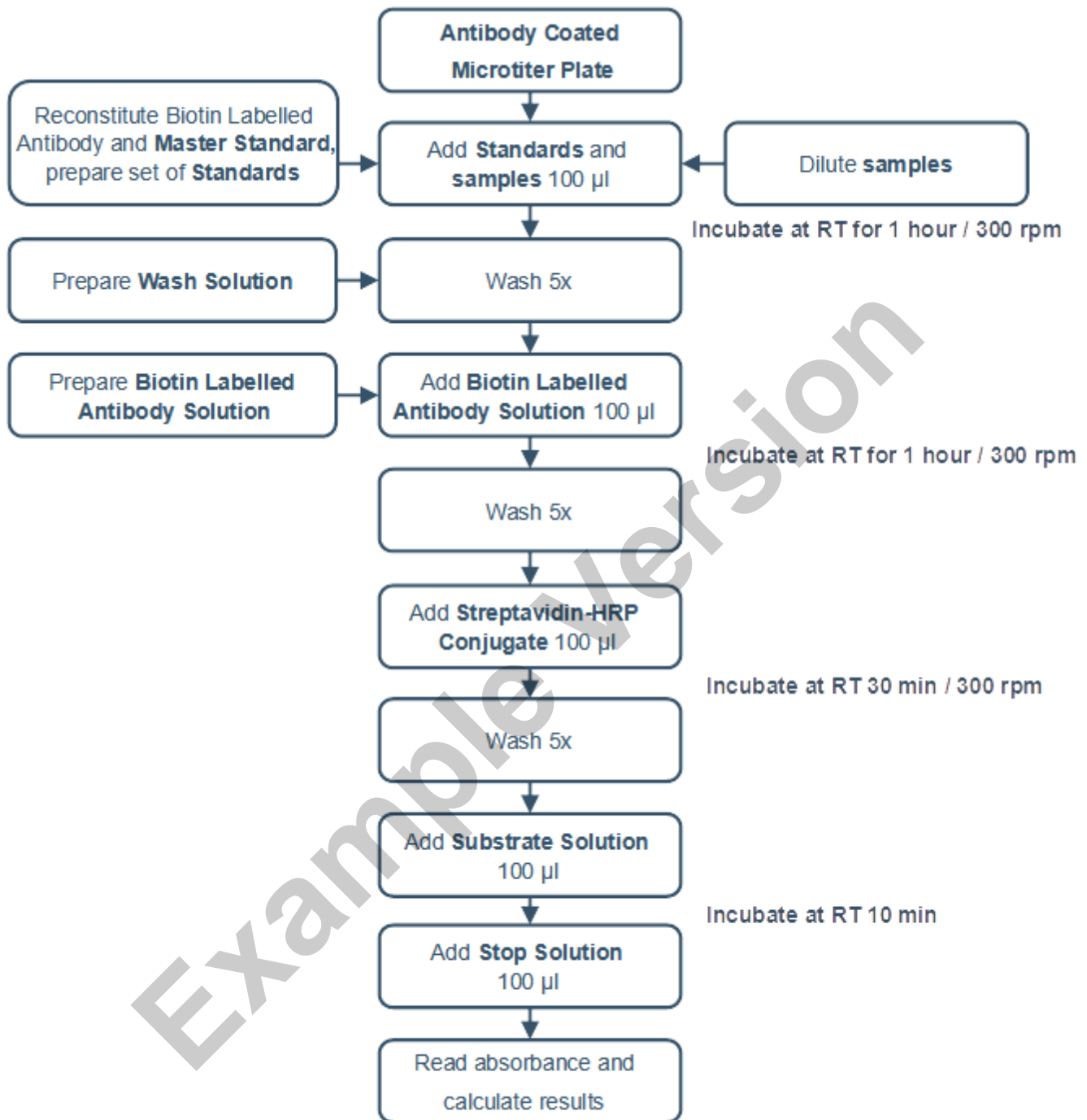
For more references on this product see our web pages at www.biovendor.com.

Example Version

19. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
 <p data-bbox="260 1184 467 1216">www.biovendor.com</p>	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

20. ASSAY PROCEDURE - SUMMARY





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