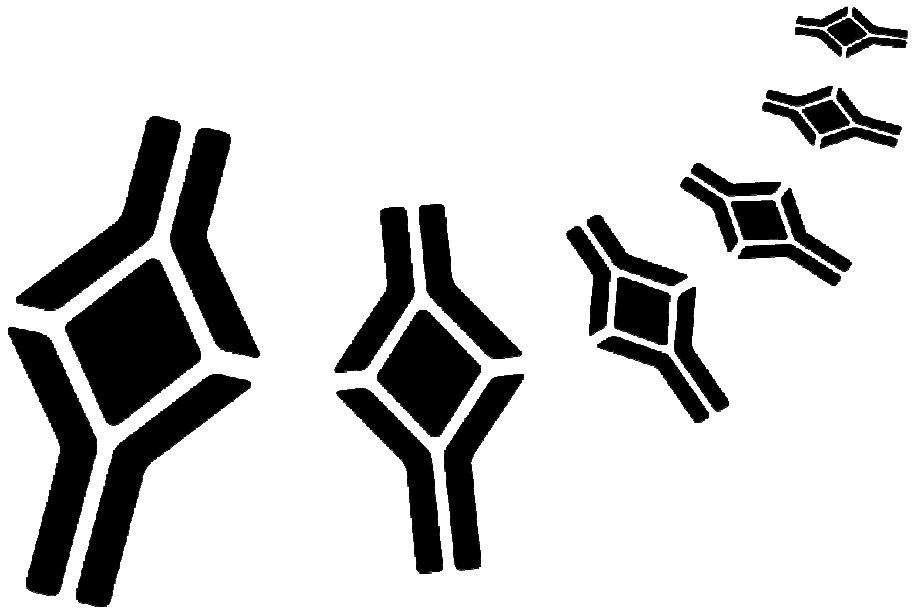

HUMAN CARTILAGE OLIGOMERIC MATRIX PROTEIN ELISA

Product Data Sheet

Cat. No.: RD194080200

European Union: IVD CE

Rest of the world:
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 RD194080200 Human Cartilage Oligomeric Matrix Protein ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human cartilage oligomeric matrix protein (COMP).

Features

- **European Union: for in vitro diagnostic use**
  Rest of the world: for research use only!
- The total assay time is less than 3.5 hours
- The kit measures COMP in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Cartilage oligomeric matrix protein (COMP), also designated thrombospondin 5 (TSP 5), is non-collagenous glycoprotein and is a member of the thrombospondin family of extracellular proteins. COMP is a calcium-binding protein of high molecular weight (>500kDa) present in the extracellular matrix of articular, nasal and tracheal cartilage. COMP is not only cartilage-derived but was found widely in other tissues, including synovium and tendon.
Intact COMP is pentameric, with five identical subunits and the carboxy-terminal globular domain of native COMP binds to collagens I, II, and IX. It has been proposed that COMP molecules are important for maintaining the properties and integrity of collagen network. In addition COMP may have a storage and delivery function for hydrophobic cell-signaling molecules such as vitamin D. The significance of COMP for normal development and function of cartilage has been underscored by the discovery that mutations of the COMP gene result in pseudoachondroplasia and some forms of multiple epiphyseal dysplasia.
Most published studies have shown that serum levels of COMP provide important information about metabolic changes occurring in the cartilage matrix in joint disease. These studies describe that serum COMP level correlated with cartilage degradation and is a potential prognostic marker in inflammatory joint diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA). Results have demonstrated an association of increasing serum COMP levels with progressive destruction of articular cartilage monitored radiographically. OA and RA are a common disease causing pain and disability in a significant proportion of the adult population and early diagnostics of these diseases is very important for future therapy.

<u>Areas of investigation:</u>
Joint diseases
Osteoarthritis
Rheumatoid arthritis

4. TEST PRINCIPLE

In the BioVendor Human Cartilage Oligomeric Matrix Protein ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human COMP antibody. After 60 minutes incubation and washing, biotin labelled second monoclonal anti-human COMP antibody is added and incubated with captured COMP 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 COMP. 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
Biotin Labelled Antibody	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	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
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 550-650 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Biotin Labelled Antibody
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 COMP 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 COMP in the stock solution is **128 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	128 ng/ml
300 µl of stock	300 µl	64 ng/ml
300 µl of 64 ng/ml	300 µl	32 ng/ml
300 µl of 32 ng/ml	300 µl	16 ng/ml
300 µl of 16 ng/ml	300 µl	8 ng/ml
300 µl of 8 ng/ml	300 µl	4 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Do not store the Standard stock solution 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).
Reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
The reconstituted Quality Controls must be used immediately or stored frozen at -20°C for 1 month. Avoid repeated freeze/thaw cycles.

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 human COMP in serum and plasma (EDTA, citrate, heparin).

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 samples 50x with Dilution Buffer just prior to the assay (e.g. 5 µl of sample + 245 µl of Dilution Buffer for singlets or duplicates). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C for long-term storage. 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 human COMP.

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, Quality Controls, 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 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 Biotin Labelled Antibody 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 Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note: If 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 COMP 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 128**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 64**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 32**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 16**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 8**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 4**	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 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 COMP 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. 20 ng/ml (from standard curve) x 50 (dilution factor) = 1 000 ng/ml.

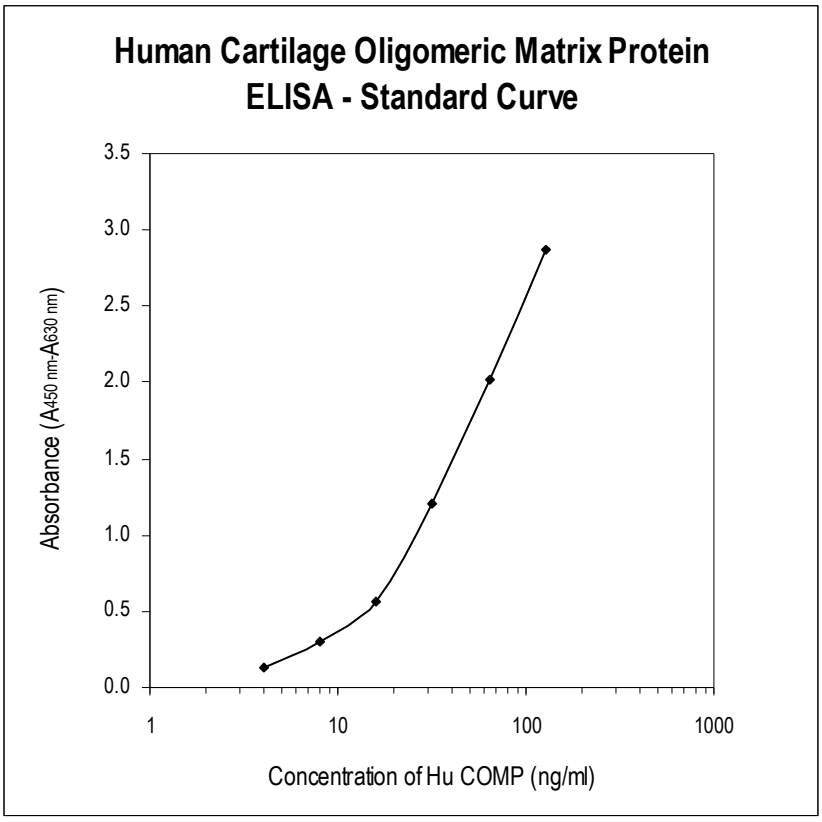

Figure 2: Typical Standard Curve for Human Cartilage Oligomeric Matrix Protein ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Cartilage Oligomeric Matrix Protein 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 human COMP values in wells and is 0.4 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding COMP level of 128 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the COMP concentration.

- **Specificity**

The antibodies used in this ELISA are highly specific for human COMP.
Determination of COMP does not interfere with hemoglobin (1mg/ml), bilirubin (170 µmo/l) and triglycerides (5,0 mmol/l).

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	495	18.5	4.0
2	1 290	102.5	8.0

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	625	19.1	3.1
2	1 604	105.1	6.6

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	495	-	-
	2 200	2 495	88.2
	1 405	1 495	94.0
	910	995	91.5
2	1 220	-	-
	3 630	3 220	112.7
	2 230	2 220	100.5
	1 700	1 720	98.8

- **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	-	1 817	-	-
	2x	937	909	103.9
	4x	500	454	105.4
	8x	207	227	83.7
2	-	3 970	-	-
	2x	1 940	1 985	97.7
	4x	990	992	99.7
	8x	420	496	84.6

- **Effect of sample matrix**

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

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	480	303	410	498
2	1 265	530	880	1 090
3	1 593	810	1 010	1 460
4	1 070	510	630	995
5	1 105	640	860	1 060
6	2 115	1 240	1 655	2 205
7	1 040	639	710	1 065
8	705	340	475	700
9	980	395	725	1 005
10	380	280	295	360
Mean (ng/ml)	**1 073**	**569**	**765**	**1 044**
Mean Plasma/Serum (%)	**-**	**53**	**71**	**97**
Coefficient of determination R^2	**-**	**0.90**	**0.95**	**0.98**

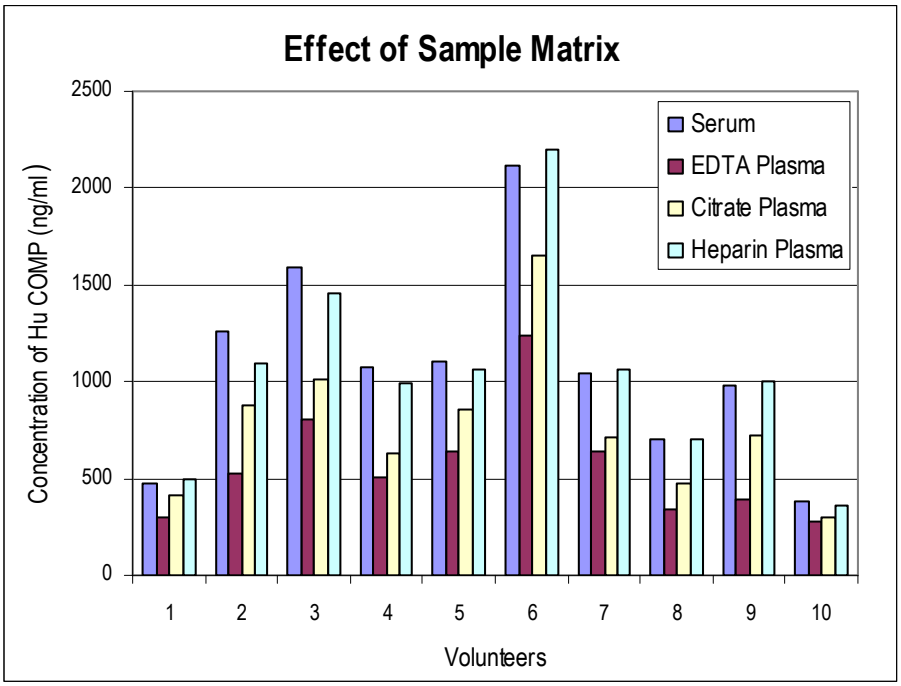

Figure 3: COMP levels measured using Human Cartilage Oligomeric Matrix Protein ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at –20°C. However, no decline in concentration of COMP 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 sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	*Incubation Temp, Period*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	443	328	352	488
	2-8°C, 1 day	481	302	355	499
	2-8°C, 7 days	482	264	345	374
2	-20°C	985	674	711	910
	2-8°C, 1 day	993	631	728	837
	2-8°C, 7 days	1 024	601	771	1 036
3	-20°C	847	491	629	713
	2-8°C, 1 day	866	515	654	793
	2-8°C, 7 days	905	493	605	853

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human COMP 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)*		
			EDTA	*Citrate*	*Heparin*
1	1x	1 105	632	820	1 121
	3x	1 040	610	783	914
	5x	1 010	559	727	867
2	1x	942	702	733	921
	3x	893	665	707	764
	5x	894	716	614	728
3	1x	1 224	884	1 133	1 100
	3x	1 275	843	975	1 182
	5x	1 220	856	873	1 080

14. DEFINITION OF THE STANDARD

The recombinant human COMP is used as the Standard. The recombinant human COMP is produced in cell line HEK293.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 246 unselected donors (165 female + 81 male) 7-92 years old were assayed with the Biovendor Human Cartilage Oligomeric Matrix Protein ELISA in our laboratory.

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

Sex	*Age (years)*	*n*	*Mean*	*SD*	*Min.*	*Max.*
			COMP (ng/ml)			
Women	7-19	7	257	98	93	414
	20-29	25	488	268	255	1 329
	30-39	20	487	206	204	888
	40-49	24	621	269	270	1 299
	50-59	22	867	407	408	1 884
	60-69	29	1 018	429	423	2 111
	70-92	38	1 091	527	432	3 250
Men	7-19	5	453	314	180	987
	20-29	9	523	221	234	939
	30-39	8	530	212	249	963
	40-49	15	763	268	260	1 242
	50-59	13	915	320	516	1 911
	60-69	20	925	272	519	1 551
	70-92	11	1 136	244	729	1 620

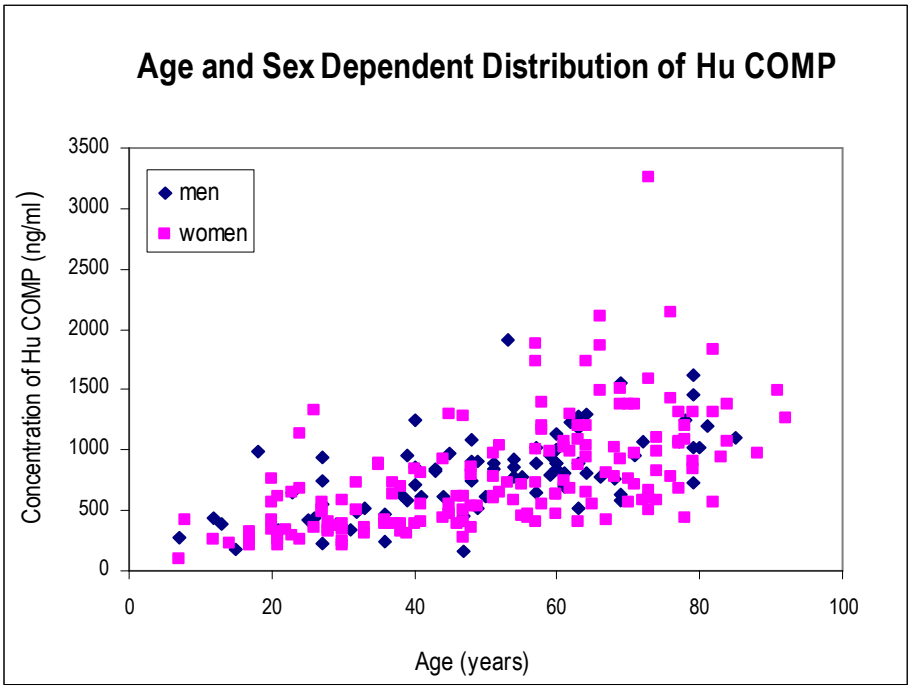

Figure 4: COMP concentration plotted against donor age.

- **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 sample in the assay. Each laboratory should establish its own normal and pathological references ranges for COMP levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Cartilage Oligomeric Matrix Protein ELISA was compared to the other commercial immunoassay, by measuring 35 serum samples. The following correlation graph was obtained:

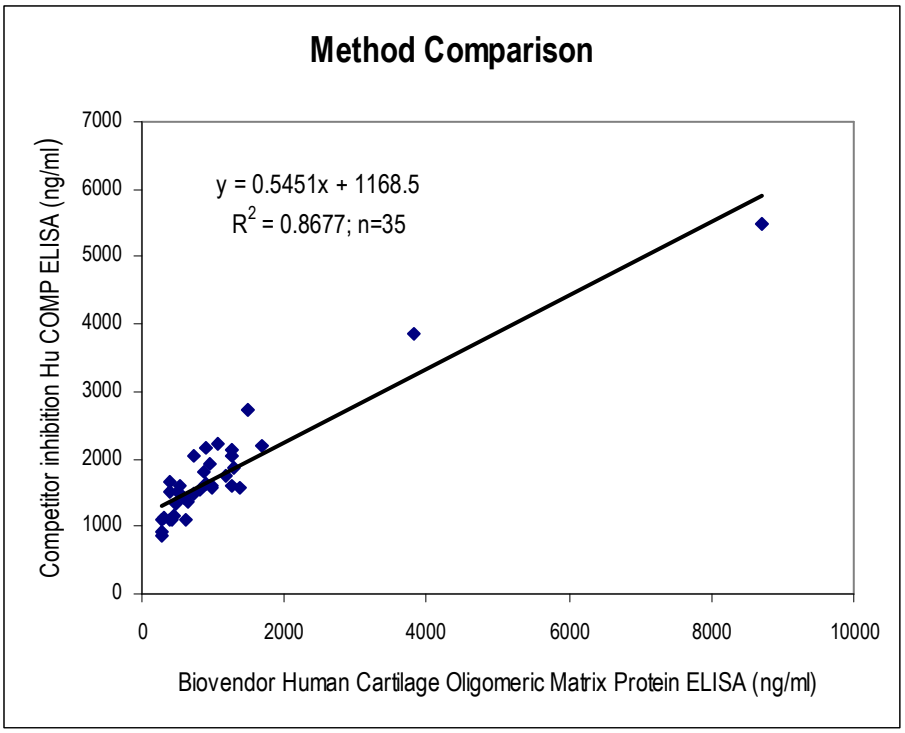

Figure 5: Method comparison.

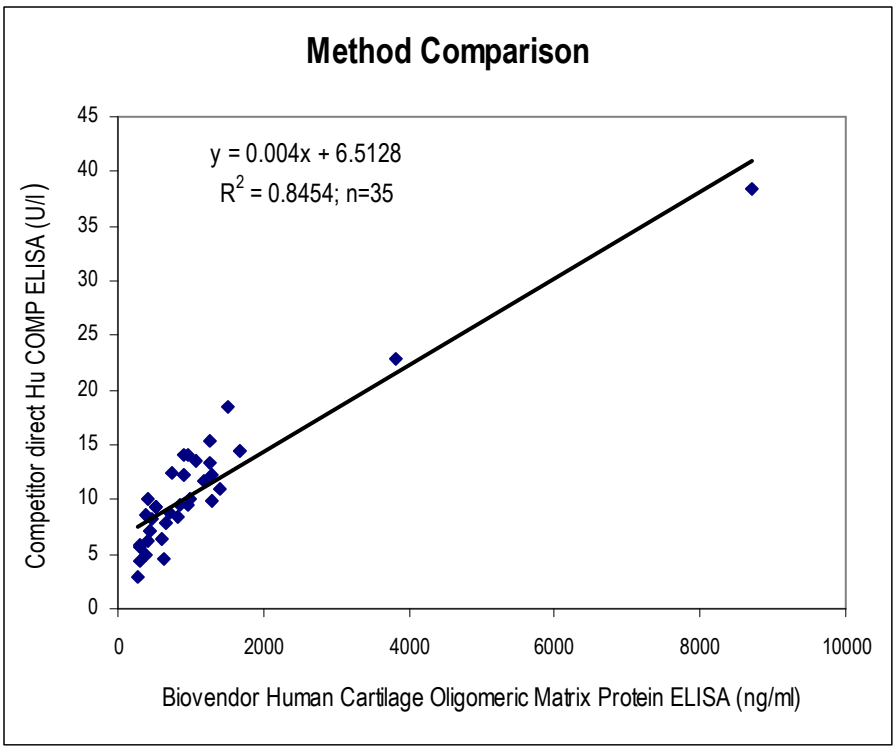

Figure 6: Method comparison.

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

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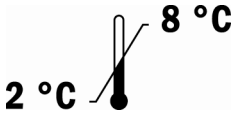
- M. Skoumal, G. Haberhauer, J. Feyertag, E.M. Kittl, K. Bauer and A. Dunky: Serum levels of cartilage oligomeric matrix protein are elevated in rheumatoid arthritis, but not in inflammatory rheumatic diseases such as psoriatic arthritis, Raynaund´s syndrome, scleroderma, systemic lupus erythematosus, vasculitis and Sjögren´s syndrome. Arthritis Res Ther 6, 73-74 (2004)

References to this product:

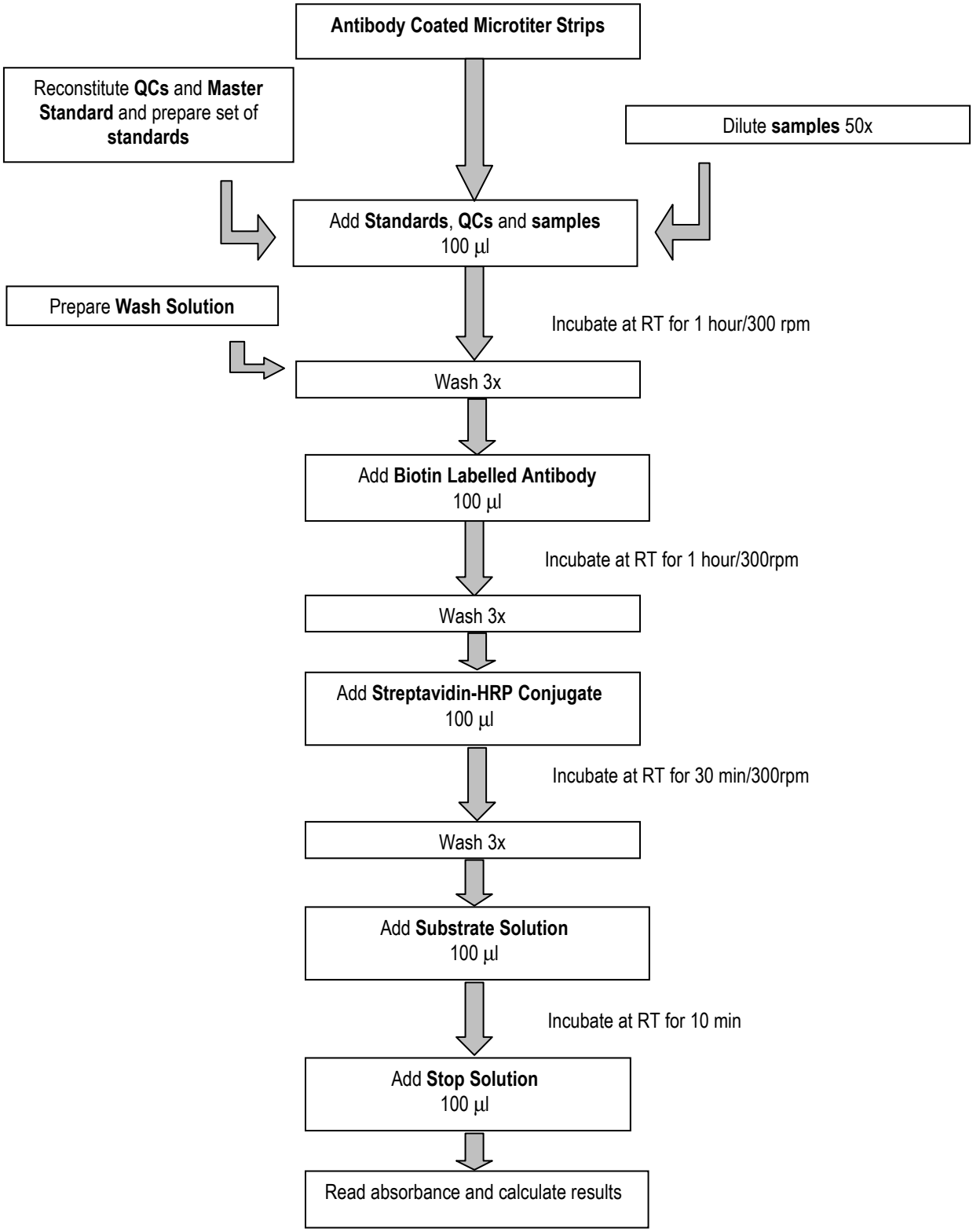
- V. Chandran, R.J. Cook, J. E.dwin, H. Shen, F.J. Pellett, S. Shanmugarajah, C.F. Rosen and D. D. Gladman: Soluble biomarkers differentiate patients with psoriatic arthritis from those psoriasis without arthrithis. Rheumatology 49, 1399–1405 (2010)

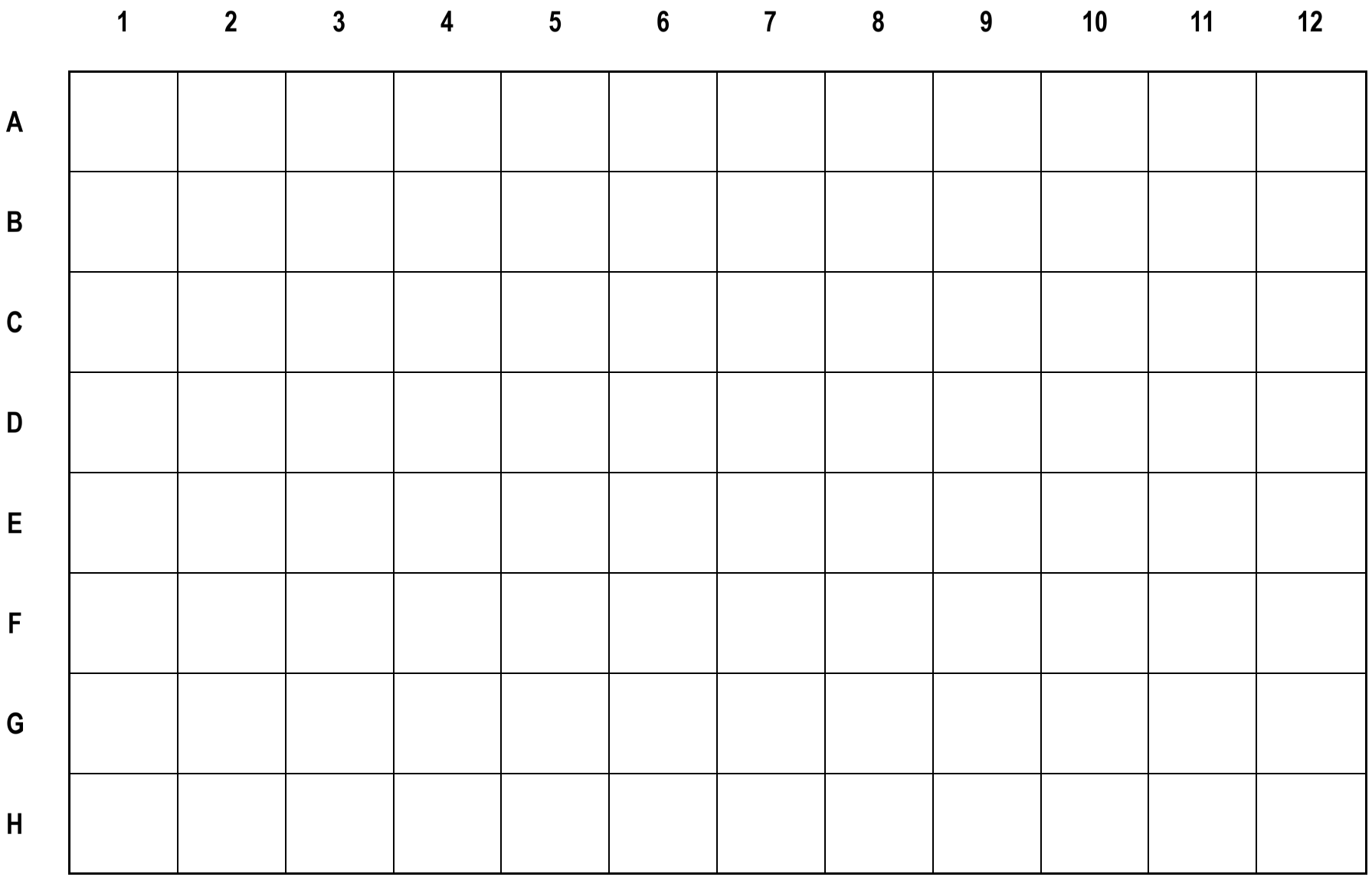
For more references on this product see our WebPages at www.biovendor.com

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
IVD CE	In vitro diagnostic medical device

Assay Procedure Summary

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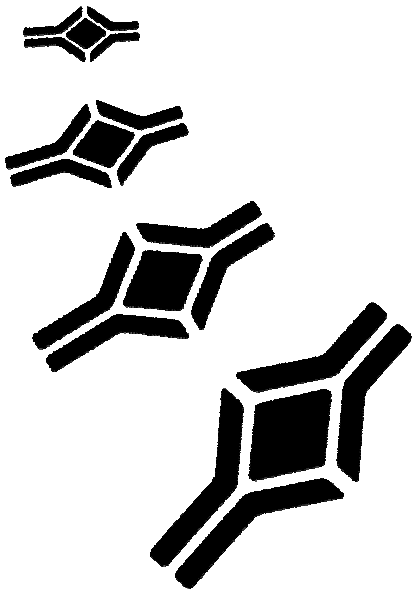

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