

Human Cartilage Oligomeric Matrix Protein ELISA

Cat. No.: RD194080200R

Manufacturer

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Use only the actual version of Product Data Sheet enclosed with the kit!

1. Intended Use

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

Features

- It is intended 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).
- Standard is recombinant protein based.
- Quality Controls are human serum based
- Components of the kit are provided ready to use, concentrated and lyophilized.

2. Storage, Expiration

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

3. Summary

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.

4. Test Principle

In the Biovendor Human Cartilage Oligomeric Matrix Protein ELISA, Standards, Quality Controls and diluted samples are incubated in microtitration wells coated with monoclonal anti-human COMP antibody. After 60 minutes and washing, biotin-labelled second monoclonal anti-human COMP antibody is added and incubated with the 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of COMP. A standard curve is constructed by plotting absorbance values versus COMP concentrations of Standards, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- For research use only.
- 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.
- Wear gloves, eyes and clothing protection when handling supplied immunodiagnostic material, particularly acidic Stop Solution, and Substrate Solution that contains hydrogen peroxide and tetramethylbenzidine (TMB). Avoid contact with these two latter reagents. The Stop and/or Substrate Solutions may cause skin/eye irritation. In such a case, wash the skin/eyes thoroughly with water and seek medical attention if necessary.
- All solutions supplied must not be pipetted by mouth.
- Do not drink, eat or smoke in the area where immunodiagnostic materials are being handled.
- Do not mix reagents from different kit lots.
- Reagents should not be used beyond the expiration date marked on the kit label.

6. Reagents Supplied

<i>Cat. No.</i>	<i>Kit Components</i>	<i>Quantity</i>
C381211	Microtiter Strips coated with Antibody, sealed	96 wells
C382611	Biotin-Labelled Antibody	13 ml
C382341	Streptavidin-HRP Conjugate	13 ml
C383241	Master Standard, lyophilized	2 vials
C384151	Quality Control High, lyophilized	2 vials
C384251	Quality Control Low, lyophilized	2 vials
C005111	Dilution Buffer, ready to use	2 x 13 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H ₂ SO ₄), ready to use	13ml
-	Product Data Sheet + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 50-200 µl
- Microplate reader with 450 ± 10 nm filter
- Orbital microplate shaker capable of agitation at approximately 300 rpm
- Software package facilitating data generation and analysis
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

8. Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

- If you do not use the whole plate, return unused strips in the provided aluminium bag with desiccant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Assay reagents supplied ready-to-use:

Microtiter Strips coated with Anti-human COMP Antibody

Biotin-labelled Anti-human COMP Antibody

Dilution Buffer

Straptavidin-HRP Conjugate

Substrate Solution (TMB)

Stop Solution (0.2M H₂SO₄)

Assay reagents supplied concentrated and lyophilized:

Wash Solution:

Dilute 100 ml of Wash Solution Concentrate (10x) with 900 ml deionized (distilled) water to prepare 1000 ml of Wash Solution (1x).

Stability and storage:

Diluted Wash Solution (1x) is stable for 1 month when stored at 2-8°C.

Human COMP Standards:

Reconstitute COMP Master Standard with 0.6 ml of Dilution Buffer, let it dissolve at least 15 minutes and mix thoroughly. The resulting concentration of the COMP in the stock solution is 128 ng/ml. Prepare other standards as follows:

<i>Standard Volume</i>	<i>Dilution Buffer</i>	<i>Standard Concentration</i>
stock	-----	128 ng/ml
300 µl of Std. 128 ng/ml	300 µl	64 ng/ml
300 µl of Std. 64 ng/ml	300 µl	32 ng/ml
300 µl of Std. 32 ng/ml	300 µl	16 ng/ml
300 µl of Std. 16 ng/ml	300 µl	8 ng/ml
300 µl of Std. 8 ng/ml	300 µl	4 ng/ml

Stability and storage: Do not store reconstituted stock solution of COMP Master Standard and other standards.

Quality Controls:

Add 0.6 ml of Dilution Buffer to the vials containing lyophilized Quality Controls Low and High, let it dissolve at least 15 minutes and mix thoroughly.

Refer to the Certificate of Analysis for actual Quality Controls values.

Stability and storage: The reconstituted quality controls have to be used immediately or to be stored frozen at -20°C until next use one month.

9. Preparation of Samples

Serum or plasma can be used as samples in BioVendor Human Cartilage Oligomeric Matrix Protein Assay.

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 serum or plasma samples.

Dilute samples 50x with Dilution Buffer just prior to performing the test, e.g. 5 µl of sample + 245 µl of Dilution Buffer and mix well (for duplicates or singlets).

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage.

For stability of samples stored at 2-8°C and effect of freezing/thawing on the COMP concentration see Chapter 13.

Do not store the diluted samples.

10. Assay Procedure

- 1) Pipet 100 μ l of Standards, Quality Controls, 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 300 rpm on an orbital microplate shaker.
- 3) Wash the wells 3-times with Wash Solution (0.35 ml per well).
- 4) Add 100 μ l of Biotin-Labelled Anti-human COMP Antibody solution into each well.
- 5) Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at 300 rpm on an orbital microplate shaker.
- 6) Wash the wells 3-times with Wash Solution (0.35 ml per well).
- 7) Add 100 μ l of Streptavidin-HRP Conjugate solution.
- 8) Incubate the plate at room temperature (ca. 25°C) for 30 min, shaking at 300 rpm on an orbital microplate shaker.
- 9) Wash the wells 3-times with Wash Solution (0.35 ml per well).
- 10) Add 100 μ l of Substrate Solution. (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). No shaking!
- 12) Stop the colour development by adding 100 μ l of Stop Solution.
- 13) Determine the absorbance by reading the plate at 450 nm. (The absorbance should be read within 10 minutes following step 12.)

Note 1: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, 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 samples. The readings at 405 nm should not replace the on-scale readings 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 absorbent paper or 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 work sheet.

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of standards against the *log* of the known concentration (X) of standards, using the four-parameter function.

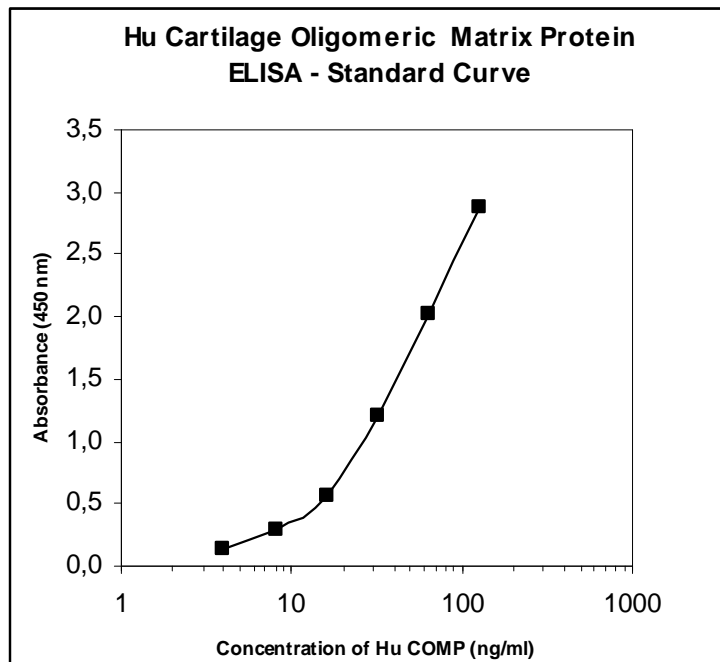


Figure 2: Standard curve for human COMP is plotted using the four-parameter function as a proportion of COMP concentration and absorbance at 450 nm.

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 COMP concentration in samples calculated from standard curve has to 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) = 1000 ng/ml.

12. Performance Characteristics

Typical analytical data obtained with the 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: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real COMP values in wells and is: 0.5 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 the Human Cartilage Oligomeric Matrix Protein ELISA are highly specific for human COMP. Sera of several mammalian species were measured in the assay. No signal has been observed when sera of following species were measured in the assay: mouse, rat, horse, bovine, sheep, goat, pig, hamster, dog, and monkey serum.

- **Precision**

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

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	CV (%)
1	645	29.0	4.5
2	1330	96.0	7.2

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>Standard Deviation (ng/ml)</i>	<i>CV (%)</i>
1	695	49.0	7.1
2	1475	66.0	4.5

- **Spiking Recovery**

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

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	525	-	-
	2300	2525	91.1
	1580	1525	103.6
	930	1025	90.7
2	740	-	-
	2410	2740	88.0
	1510	1740	86.8
	1085	1240	87.5

- **Dilution Linearity**

Serum samples were diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	2675	-	-
	2x	1390	1338	103.9
	4x	705	669	105.4
	8x	280	334	83.7
2	-	3970	-	-
	2x	1940	1985	97.7
	4x	990	992	99.7
	8x	420	496	84.6

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human COMP in serum and plasma samples after repeated (5x) freezing/thawing cycles.

Sample Number	Number of f/t cycles	Serum (ng/ml)	Plasma (ng/ml)		
			Heparin	Citrate	EDTA
1	1x	1105	1121	820	632
	3x	1040	914	783	610
	5x	1010	867	727	559
2	1x	942	921	733	702
	3x	893	764	707	665
	5x	894	728	614	716
3	1x	1224	1100	1133	884
	3x	1275	1182	975	843
	5x	1220	1080	873	856

- **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 when stored at 2-8°C for 7 days. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.3% and 0.1% concentration, respectively.

Sample Number	Incubation Temp., Period	Serum (ng/ml)	Plasma (ng/ml)		
			Heparin	Citrate	EDTA
1	-20°C	443	488	352	328
	2-8°C, 1 days	481	499	355	302
	2-8°C, 7 days	482	374	345	264
2	-20°C	985	910	711	674
	2-8°C, 1 days	993	837	728	631
	2-8°C, 7 days	1024	1036	771	601
3	-20°C	847	713	629	491
	2-8°C, 1 days	866	793	654	515
	2-8°C, 7 days	905	853	605	493

- **Effect of Sample Matrix (plasma/serum)**

Samples from 10 volunteers were taken and treated by different methods (serum, heparin, citrate, and EDTA plasma). Assay results are shown below:

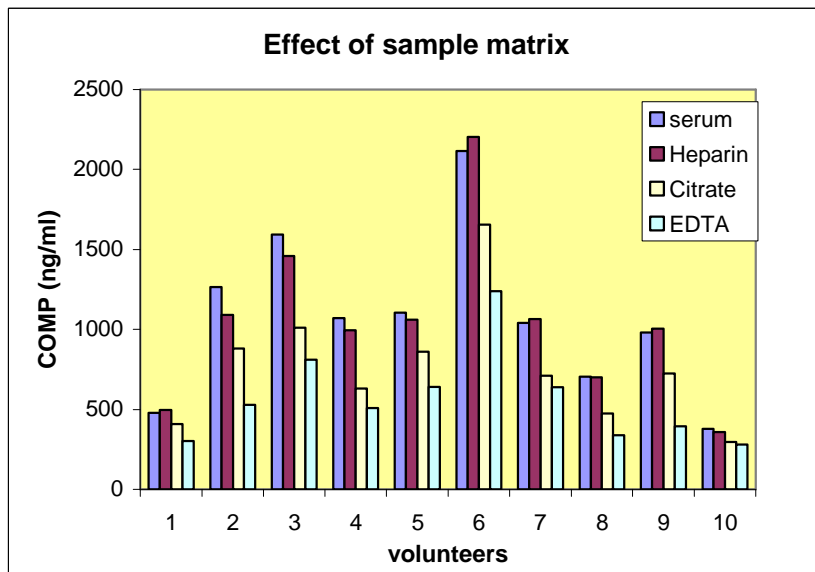


Figure 3: Ten serum samples were measured in Human Cartilage Oligomeric Matrix Protein ELISA, each with respective heparin, citrate and EDTA plasma.

Volunteer	Serum (ng/ml)	Plasma (ng/ml)		
		Heparin	Citrate	EDTA
1	480	498	410	303
2	1265	1090	880	530
3	1593	1460	1010	810
4	1070	995	630	510
5	1105	1060	860	640
6	2115	2205	1655	1240
7	1040	1065	710	639
8	705	700	475	340
9	980	1005	725	395
10	380	360	295	280
Mean	1073	1044	765	569
Mean plasma/serum	-	97%	71%	53%
Correlation coef. R²		0.98	0.95	0.90

13. Definition of the Standard

The recombinant Human COMP is used as the Standard. The recombinant Human COMP (Gln 21 – Ala 757) is produced in mouse myeloma cell line NS0.

14. Preliminary and clinical data

The following results were established when serum from 246 unselected blood donors (165 female + 81 male) 7-92 years old were assayed with the Biovondor Human Cartilage Oligomeric Matrix Protein ELISA.

Population Mean (mean +/- SEM): 790 +/- 27 ng/ml

Normal Range (mean +/- 2SD): 790 +/- 846 ng/ml

However, it is recommended to establish a normal value of control serum for each laboratory. The presented reference should be regarded as guideline.

Sex	Age years	n	Mean	SD	Min	Max
			COMP ng/ml			
Female	7-19	7	257	98	93	414
	20-29	25	488	268	255	1329
	30-39	20	487	206	204	888
	40-49	24	621	269	270	1299
	50-59	22	867	407	408	1884
	60-69	29	1018	429	423	2111
	70-92	38	1091	527	432	3250
Male	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	1242
	50-59	13	915	320	516	1911
	60-69	20	925	272	519	1551
	70-92	11	1136	244	729	1620

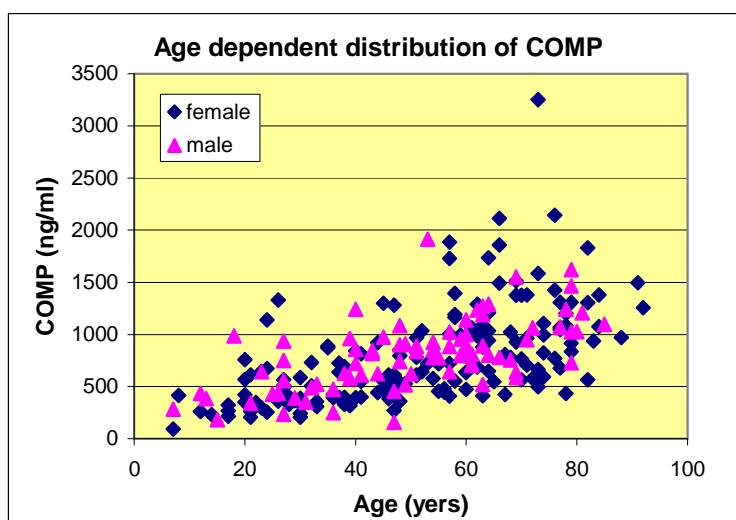
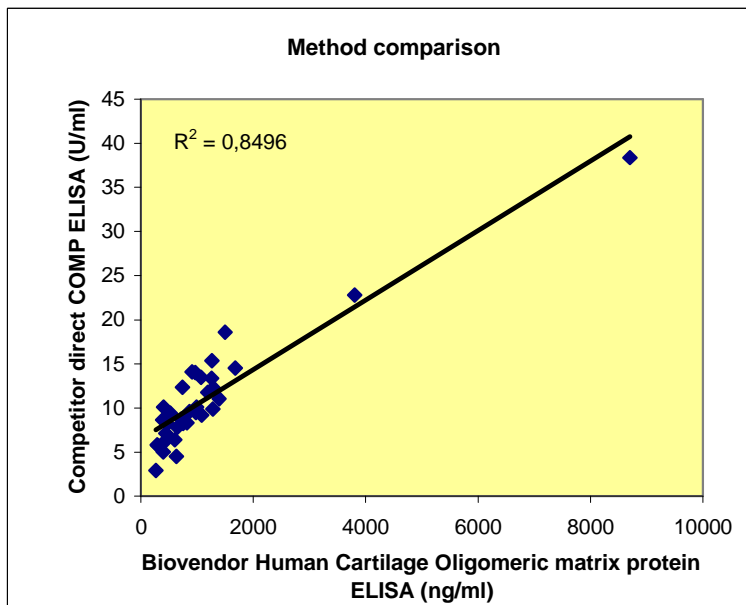
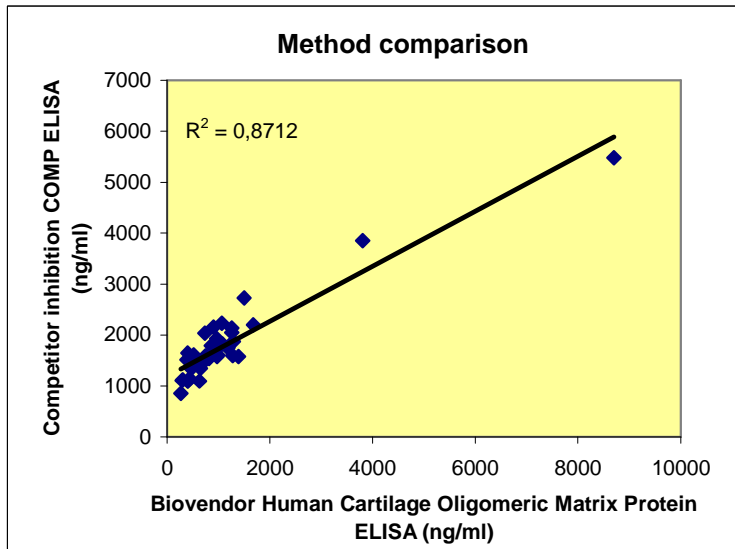


Figure 4: COMP concentration plotted against donor age.

15. Method Comparison

The BioVendor Human Cartilage Oligomeric Matrix Protein ELISA was compared to other commercial immunoassays, measuring of 35 serum samples. The following correlation graphs were obtained:



16. Troubleshooting and FAQs

1/ 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

2/ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time should be decreased before addition of Stop Solution

3/ High coefficient of variation (CV)

Possible explanation:

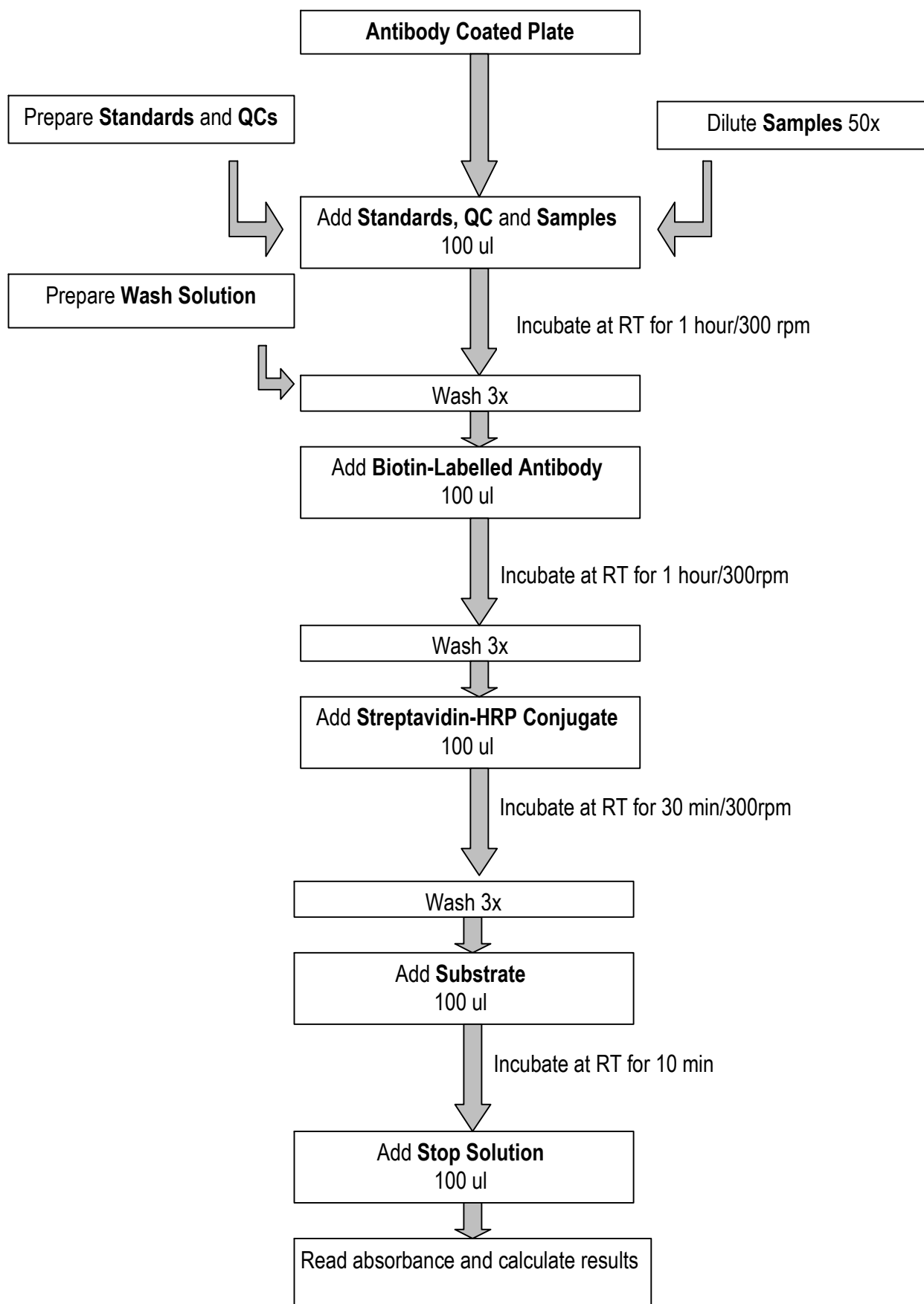
- Improper or inadequate washing

17. References

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

Assay Procedure Summary



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Notes:
