Quantikine™ ELISA

Human COMP Immunoassay

Catalog Number DCMP0

For the quantitative determination of human Cartilage Oligomeric Matrix Protein (COMP) concentrations in cell culture supernates, cell lysates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Human Cartilage Oligomeric Matrix Protein (COMP), also known as Thrombospondin-5, is a member of the Thrombospondin (TSP) protein family, which are calcium-binding extracellular glycoproteins. Based on their dimerization characteristics, the TSPs are divided into subgroups A and B. TSP-1 and TSP-2 constitute subgroup A and form homodimers, whereas TSP-3, TSP-4, and COMP constitute subgroup B and form homopentamers. COMP is a multidomain protein, which includes a non-collagenous coiled-coil domain, four EGF-like repeats, seven TSP type 3 repeats, and a globular TSP C-terminal domain. The coiled-coil domain mediates the association of COMP into disulfide-linked homopentamers of 524 kDa (1-3).

COMP is predominantly found in the extracellular matrix of cartilage, tendons, and ligaments (4). It plays a pivotal role in endochondral ossification and in the assembly and stabilization of the extracellular matrix. Mutations in the COMP gene can lead to two skeletal diseases, pseudoachondroplasia and multiple epiphyseal dysplasias, both of which are associated with severe deficiency in cartilage and bone development (5-7). It is postulated that COMP maintains the structural integrity of the cartilage through its interaction with a number of extracellular matrix proteins. COMP can bind to collagens type I and II via its C-terminal globular domain and act as a catalyst to promote fibril formation (8-9). It can also bind to aggrecan, fibronectin, and matrilin (10-12). Through the interaction with integrin, COMP can aid in the attachment of chondrocytes to cell culture dishes (13). COMP can also inhibit cell proliferation while enhancing chondrogenesis (14). Furthermore, by elevating survival proteins, COMP can protect chondrocytes from cell death (15). COMP has a unique binding site for Vitamin D, indicating that it may also participate in storage and delivery of cell-signaling molecules (16-17).

COMP has been widely studied in various types of arthritis and has been demonstrated to be a biomarker for cartilage breakdown. The highest level of COMP is detected in the synovial fluid. COMP is subjected to proteolytic cleavage in the cartilage of patients suffering from arthritis (18). These fragments are detectable in the circulation and may serve as diagnostic and prognostic indicators and as biomarkers for disease severity and response to treatment (19). For example, in the chronic erosive arthritis rat model, serum COMP levels correlate with the degree of cartilage destruction (20). In patients with osteoarthritis, serum levels of COMP are elevated and the degree of elevation seems to correlate with abnormalities observed in bone scans (21). In rheumatoid arthritis, serum COMP levels decrease in those patients who respond to treatment (22).

The Quantikine™ Human COMP immunoassay is a 4.5 hour solid phase ELISA designed to measure human COMP in cell culture supernates, cell lysates, serum, and plasma. It contains NS0-expressed recombinant human COMP and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human COMP showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for natural human COMP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human COMP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any COMP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human COMP is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of COMP bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human COMP Microplate	893889	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human COMP.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human COMP Standard	893891	2 vials of recombinant human COMP in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard for each assay. Discard after use.
Human COMP Conjugate	893890	21 mL of a polyclonal antibody specific for human COMP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-73	895541	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 100 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human COMP Controls (optional; R&D Systems®, Catalog # QC66)

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 1 (R&D Systems, Catalog # 890713)
- Phosphate-buffered saline (PBS)

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed prior to assaying. Refer to the Sample Values section for details.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

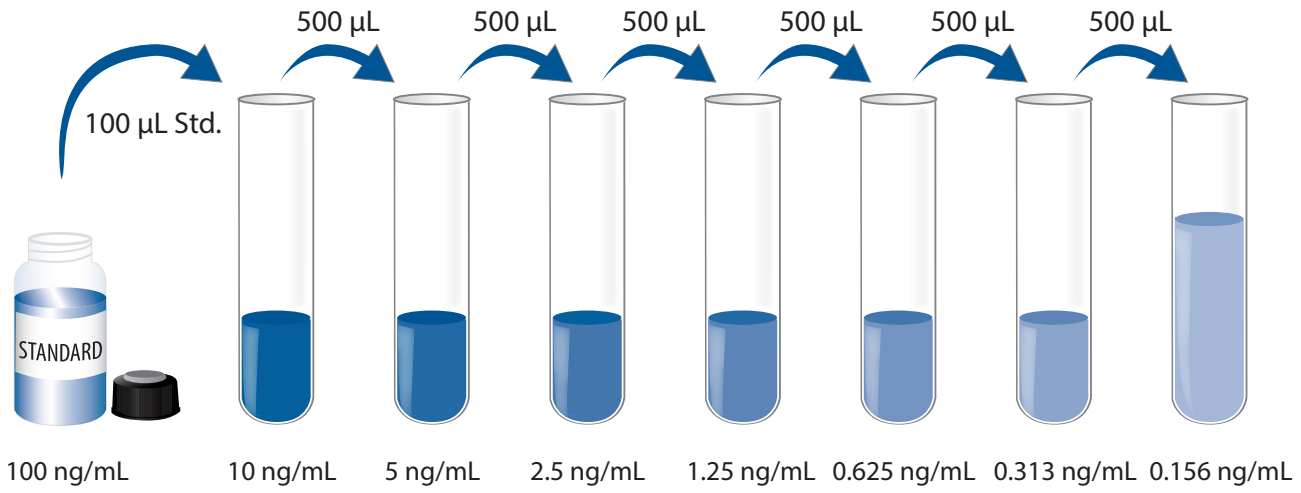
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human COMP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human COMP Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-73 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human COMP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

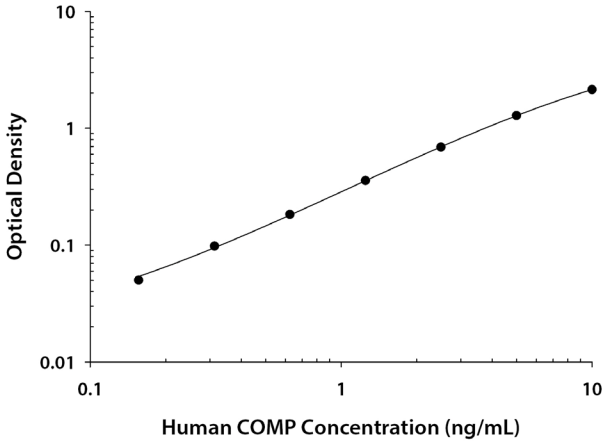
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human COMP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
0.156	0.059 0.061	0.060	0.050
0.313	0.107 0.108	0.108	0.098
0.625	0.185 0.201	0.193	0.183
1.25	0.362 0.369	0.366	0.356
2.5	0.673 0.725	0.699	0.689
5	1.244 1.341	1.293	1.283
10	2.146 2.152	2.149	2.139

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (ng/mL)	0.900	2.92	6.11	1.05	3.07	6.26
Standard deviation	0.028	0.112	0.225	0.045	0.146	0.300
CV (%)	3.1	3.8	3.7	4.3	4.8	4.8

RECOVERY

The recovery of human COMP spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	103	95-111%
Cell lysates (n=1)	102	98-108%
Serum* (n=4)	102	86-114%
EDTA plasma* (n=4)	103	86-114%
Heparin plasma* (n=4)	99	88-112%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human COMP were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Cell lysates (n=2)	Serum* (n=8)	EDTA plasma* (n=8)	Heparin plasma* (n=8)
1:2	Average % of Expected	102	100	102	102	100
	Range (%)	97-105	97-102	97-105	98-109	96-104
1:4	Average % of Expected	101	99	102	102	99
	Range (%)	91-108	97-101	97-106	97-109	95-104
1:8	Average % of Expected	97	94	103	101	98
	Range (%)	89-103	93-94	93-107	95-108	92-101
1:16	Average % of Expected	88	94	103	99	95
	Range (%)	86-93	94-94	90-115	88-108	86-101

*Sample were diluted prior to assay.

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of human COMP ranged from 0.005-0.036 ng/mL. The mean MDD was 0.010 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human COMP produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human COMP in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	171	66.8-409	76.3
EDTA plasma (n=35)	146	58.7-304	62.0
Heparin plasma (n=34)	150	60.9-339	66.0

Cell Culture Supernates:
HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin-sulfate until confluent. An aliquot was removed, assayed for human COMP, and measured 0.603 ng/mL.

BUD-8 human fibroblast cells were cultured in DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine in a T75 flask until confluent. An aliquot was removed, assayed for human COMP, and measured 19.3 ng/mL.

Cell Lysates - BUD-8 human fibroblast cells were cultured as described above. After the supernate was harvested, the cells were gently washed with cold PBS. The PBS was poured off and discarded. 10 mL of Cell Lysis Buffer 1 was added to the T75 flask and incubated at room temperature for 1 hour with gentle agitation. The cell lysate was collected and centrifuged at 12,000 rpm for 10 minutes to remove cell debris. The supernate was collected, and the total protein concentration of the cell lysate was determined with the Bradford method (23). The cell lysate was aliquoted and frozen at ≤ -70 °C until use. An aliquot was removed, assayed for human COMP, and measured 8.43 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human COMP.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human COMP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ADAMTS12
Aggrecan G1-IGD-G2 Domains
Matrilin-2
Matrilin-3
Matrilin-4
Thrombospondin-1
Thrombospondin-2
Thrombospondin-4

REFERENCES

1. Oldberg, A. *et al.* (1992) J. Biol. Chem. **267**:22346.
2. Newton, G. *et al.* (1994) Genomics **24**:435.
3. Hedbom, E. *et al.* (1992) J. Biol. Chem. **267**:6132.
4. Di Cesare, P.E. *et al.* (1994) FEBS Lett. **354**:237.
5. Briggs, M.D. *et al.* (1995) Nat. Genet. **10**:330.
6. Hecht, J.T. *et al.* (1995) Nat. Genet. **10**:325.
7. Stanescu, R. *et al.* (1993) Am. J. Med. Genet. **45**:501.
8. Rosenberg, K. *et al.* (1998) J. Biol. Chem. **273**:20397.
9. Halasz, K. *et al.* (2007) J. Biol. Chem. **282**:31166.
10. Chen, F.H. *et al.* (2007) J. Biol. Chem. **282**:24591.
11. Man, H.H. *et al.* (2004) J. Biol. Chem. **279**:25294.
12. Di Cesare, P.E. *et al.* (2002) Matrix Biol. **21**:461.
13. Chen, F.H. *et al.* (2005) J. Biol. Chem. **280**:32655.
14. Kipnes, J. *et al.* (2003) Osteoarthr. Cartil. **11**:442.
15. Gagarina, V. *et al.* (2008) J. Biol. Chem. **283**:648.
16. Malashkevich, V.N. *et al.* (1996) Science **274**:5288.
17. Ozbek, S. *et al.* (2002) EMBO J. **21**:5960.
18. Neidhart, M. *et al.* (1997) Br. J. Rheumatol. **36**:1151.
19. Posey, K. *et al.* (2008) Curr. Drug Targets **9**:869.
20. Vingsbo-Lundberg, C. *et al.* (1998) Arthritis Rheum. **41**:544.
21. Clark, A.G. *et al.* (1999) Arthritis Rheum. **42**:2356.
22. Crnkic, M. *et al.* (2003) Arthritis Res. Ther. **5**:R181.
23. Bradford, M.M. (1976) Anal. Biochem. **72**:248.