Instruction Manual No. M-100-440-COMP

Human Cartilage oligomeric matrix protein (COMP) ELISA KIT

Cat # 100-440-COMP, 96 tests

For Quantitative Determination of COMP
In Human Serum, plasma or cell culture medium

For In Vitro Research Use Only

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHANGES.

Human COMP ELISA KIT #100-440-COMP, 96 Tests

Kit Components	Cat #
Anti-hCOMP IgG coated strips, 96 wells,	100441
Human COMP, Recombinant, lyophilized standard (10 ng), 2 vials; make more standards	100442
Anti-hCOMP IgG-biotin ab #1 (100x), 130 ul	100443
Avidin-Peroxidase (HRP) conj. # 2, (100x) 130 ul (dilute 1:100 with water before use)	100444
Sample diluent buffer, 30 ml	100440-SD
Antibody Diluent buffer for ab #1, 12 ml	100440-AD
ABC Diluent buffer for conjugate #2, 12 ml	100440-ABC
Wash buffer (20X), 25 ml (dilute 1:20 with distilled water, **25 ml stock to 475 ml dH2O**)	100440-WB
TMB Substrate Solution, 10 ml	100440-TMB
Stop solution, 10 ml	100440-SS
Complete Instruction Manual,	M-100-440-comp

Intended use

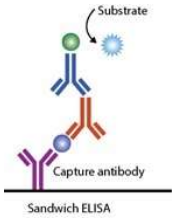
Human Cartilage oligomeric matrix protein (COMP) is a sandwich ELISA for quantitative detection of human COMP in cell culture supernates (culture medium), serum and plasma (heparin, EDTA, citrate). For research use only (RUO), not for diagnosis, cure or prevention of the disease.

Introduction

Cartilage oligomeric matrix protein (COMP) is a protein that in humans is encoded by the COMP gene. The sequences of rat and bovine COMP indicate that it is a member of the thrombospondin gene family. By Southern blot analysis of a somatic cell hybrid DNA panel and by isotopic in situ hybridization, human COMP gene was mapped to 19p13.1. COMP is a marker of cartilage turnover. COMP play a role in the structural integrity of cartilage via its interaction with other extracellular matrix proteins such as the collagens and fibronectin. Can mediate the interaction of chondrocytes with the cartilage extracellular matrix through interaction with cell surface integrin receptors. Could play a role in the pathogenesis of osteoarthritis. Potent suppressor of apoptosis in both primary chondrocytes and transformed cells. Suppresses apoptosis by blocking the activation of caspase-3 and by inducing the IAP family of survival proteins (BIRC3, BIRC2, BIRC5 and XIAP). Essential for maintaining a vascular smooth muscle cells (VSMCs) contractile/differentiated phenotype under physiological and pathological stimuli. Maintains this phenotype of VSMCs by interacting with ITGA7 (By similarity).

Tissue Specificity Abundantly expressed in the chondrocyte extracellular matrix, and is also found in bone, tendon, ligament and synovium and blood vessels. Increased amounts are produced during late stages of osteoarthritis in the area adjacent to the main defect.
Sequence Similarities Belongs to the thrombospondin family.
Subcellular Localization Secreted, extracellular space, extracellular matrix.
Uniprot ID COMP: P49747
Alternative Names Cartilage oligomeric matrix protein | COMP | EDM 1 | EDM1 | P49747 | PSACH | Pseudoachondroplasia | Thrombospondin5 | Thrombospondin-5 | TSP5

PRINCIPLE OF THE TEST



Human COMP ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for COMP has been precoated onto 96-well plates. Standards and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for COMP is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human COMP amount of sample captured in plate.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 µl) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The COMP ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions.
Applicable **MSDS**, if not already on file, **for** the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

1. Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell Lysates**: After sufficient splitting, there should be no obvious cell sediment .Centrifuge cell lysates at approximately 10000 X g for 5 min. Collect the cell lysate supernates to go ahead
- **Cell culture supernates**: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.
- **Plasma**: Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 20 min at 2000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.
- **Tissue**: Put the fresh tissues in prechill physiological saline quickly, rinse several times. Take the tissues out, cut up and put them into homogenizer. Add lysate solution to the tissues in 10:1 (lysate solution: tissue net weight = 10:1, i.e. Add 10ml lysate solution to 1g tissues), then, homogenize, centrifugate and collect the supernatant (Ultrasounding if there is dope).

2. Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

- **High target protein** concentration (100-1000 ng/ml). The working dilution is 1:100. i.e. Add 1µl sample into 99 µl sample diluent buffer.
- **Medium target protein** concentration (10-100 ng/ml). The working dilution is 1:10. i.e. Add 10µl sample into 90 µl sample diluent buffer.

- **Low target protein** concentration (10-100 ng /ml). The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein** concentration (≤10 ng/ml). No dilution necessary, or the working dilution is 1:2.

3. Reagent Preparation and Storage

A. Reconstitution of the human COMP standard:

COMP standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of COMP standard (10ng or 10,000 pg per tube) are included in each kit. Use one tube for each experiment.

Prepare stock standard of 10,000 pg/ml of human COMP (Stock or Std F): Add 1 ml sample diluent buffer into one tube, mix gently and leave the tube at room temp. for 10 min and. Do not vortex vigorously.

Stds	Sample diluent	Total volume	**Final Conc pg/ml**
Stds E-A are prepared by 2-fold serial dilutions			
0.2 ml of Std F, 10000 pg/ml	0.20 ml	0.4 ml	Std E 5000 pg/ml
0.2 ml of Std E, 5000 pg/ml	0.20 ml	0.4 ml	Std D, 2500 pg/ml
0.2 ml of Std D 1250 pg/ml	0.20 ml	0.4 ml	Std C, 1250 pg/ml
0.2 ml of Std C, 625 pg/ml	0.20 ml	0.4 ml	Std B, 312 pg/ml
0.2 ml of Std B, 312 pg/ml	0.20 ml	0.4 ml	Std A, 156 pg/ml

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. **Preparation of biotinylated anti-human COMP antibody working solution**:

The solution should be prepared no more than 2 hours prior to the experiment.

i) The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume.

ii) Biotinylated anti-human COMP antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 10 µl Biotinylated anti-human COMP antibody to 990 µl antibody diluent buffer.)

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution:

The solution should be prepared no more than 1 hour prior to the experiment.

i) The total volume should be: 0.1 ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)

D. **Avidin- Biotin-Peroxidase Complex (ABC**) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1µl ABC to 99µl ABC diluent buffer.)

E. Preparation of 1X wash buffer: Dilute wash buffer (1:20) with distilled water (25 ml stock to 475 ml of distilled or deionized water). Store at 4oC.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells.

Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate.

The unused strips should be stored in a sealed bag at 4°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. Once opened/used standards are stable for two month at 2-8°C. The unused portions of the standards should be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE

Prepare standards, samples, and all reagents before using the kit. The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard COMP detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of COMP amount in samples.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. **Add 0.1ml per well of the standards** (5000, 2500, 1250, 625,, 312 and 156 pg/ml hCOMP) into the coated wells. Add 0.1ml of the sample diluent buffer into the control well (blank or Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates, serum or plasma(heparin, EDTA, citrate) to each empty well. **See "Sample Dilution Guideline" above for details.** We recommend that each human COMP standard solution and each sample is measured in duplicate.

2. Seal the plate with the cover and **incubate at 37°C for 90 min**.

3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. **Add 0.1ml of 1x biotinylated anti-human COMP antibody** working solution into each well and incubate the plate at **37°C for 60 min**.

5. **Wash plate 3 times** with 1x wash buffer (300 ul/well), each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. Repeat this process two additional times for a total of THREE washes. **Note**: For automated washing, aspirate all wells and wash THREE times with 300 ul wash buffer. Blot the plate onto paper towels or other absorbent material.)

6. **Add 0.1ml of prepared 1X ABC working solution** into each well and **incubate the plate at 37°C for 30 min**.

7. **Wash plate 5 times** as in step 5.

8. **Add 90 µl of prepared TMB color** developing agent into each well and incubate plate at **37°C in dark for 20-25 min** (Note: blue color develops in 4 highest standards and positive samples. **T**he other wells show no obvious or intense color). Note: TMB incubation time may be varied 5-10 mins so as to get the highest standards A450 reading of 2.00-3.00 or within the linear reading range of most ELISA readers.

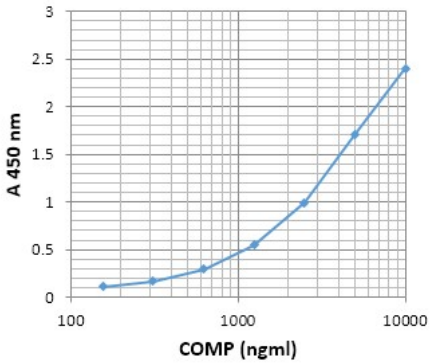
9. **Add 0.1 ml of prepared TMB stop solution into each well**. The color changes into yellow immediately. :

10. **Read the plates 450nm/630nm** (reference filter) in an ELISA reader within 30 min after adding the stop solution.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	A450 Net Abs.
A1, A2	**blank** (0 pg/ml)	0.03
B1, B2	**Std. A** (156 pg/ml)	0.107
C1, C2	**Std. B** (312 pg/ml)	0.168
D1, D2	**Std. C** (625 pg/ml)	0.29
E1, E2	**Std. D** (1250 pg/ml)	0.55
F1, F2	**Std. E** (2500 pg/ml)	0.99
G1, G2	**Std. F** (5000 pg/ml)	1.7
H1, H2	**Std. G** (10,000 pg/ml)	2.4

NOTE: These data are for demonstration purpose only. A complete std. curve must be run in every assay to determine sample values.



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A typical std curve (Plot linear graph Draw the best curve through the points. Do not use this for calculating sample values).

Calculations

Subtract the A450 values of blank or zero wells from all values. Plot A450 values of the standard on (Y) axis vs. concentration (X) on a semilog scale. The sample TNF-alpha concentration can be interpolated from the standard curve. **Note:** if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Performance Characteristics

Range

156-10,000pg/ml

Sensitivity

< 10 pg/ml
Sensitivity, or Lower Limit of Detection (LLD), is the minimum level of target protein the ELISA assay can detect. We measure 20 blank wells and if the O.D. value is 2 standard deviations higher than the blanks' average O.D. the sample can be deemed positive

Specificity

Natural and recombinant human COMP

Cross-reactivity
No detectable cross-reactivity with other relevant proteins

Species cross-reactivity
The antibodies (anti-human COMP) used this kit has not been tested for potential cross-reactive with COMP from species like monkey, mouse, and rat etc. ADI has separate ELISA kits for mouse and rat COMP.

Intra/Inter Assay Precision

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Intra-Assay Precision				Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(ng/ml)	1.02	3.12	6.19	1.13	3.08	6.23
Standard deviation	0.052	0.131	0.254	0.07	0.166	0.324
CV(%) 5.1	4.2	4.1	6.2	5.4	5.2	

Quick Summary of COMP ELISA

1. Add **0.1 ml samples and standards and incubate the plate at 37°C for 90 min**. Do not wash.
2. **Add 0.1 ml 1X biotin antibodies** and **incubate the plate at 37°C for 60 min**.
3. **Wash plate 3 times** with wash buffer (300 ul/wash).
4. **Add 0.1 1X ABC solution** and **incubate the plate at 37°C for 30 min**.
5. **Wash plate 5 times** with wash buffer
6. Add **90 ul TMB and incubate the plate at 37°C in dark for 20-25 min**.
7. Add **100 TMB stop solution** and **read at 450nm/630nm**.

References: Steikesserer A (1992) Genomics 13, 654-657; Patterson D (1993) Genomics 15, 173-176;

ELISA kits available from ADI (see details at the web site)

200-800-AVG **Avastin/Bevacizumab (Anti-TNF-alpha) ELISA Kit for human, 96 tests**
200-810-ADG **Human Anti-Avastin/Bevacizumab IgG (anti-drug IgG) ELISA Kit for human, 96 tests**
200-820-VEF **Human TNF-alpha ELISA Kit, 96 tests**
200-830-VEM **Mouse TNF-alpha ELISA Kit, 96 tests**
200-840-VER **Rat TNF-alpha ELISA Kit, 96 tests**
200-850-FLT **Human TNF-alphaR1/FLT1 ELISA Kit, 96 tests**
200-860-KDR **Human TNF-alphaR2/KDR ELISA Kit, 96 tests**
200-870-ID24 **Avastin/Bevacizumab identification/Counterfeit detection ELISA Kit, 24 tests**

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin **#1200** Human Albumin (Urinary)
#1750 Human IgG (total) **#1760** Human IgM
#1800 Human IgE **#1810** Human Ferritin
#1210 Human Transferrin (Tf) **#0020** Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) **#1830** Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) **#1510** free PSA (fPSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin **#0040** Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #**0410** HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) **#1110** Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) **#1700** Human T3 (total)

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA