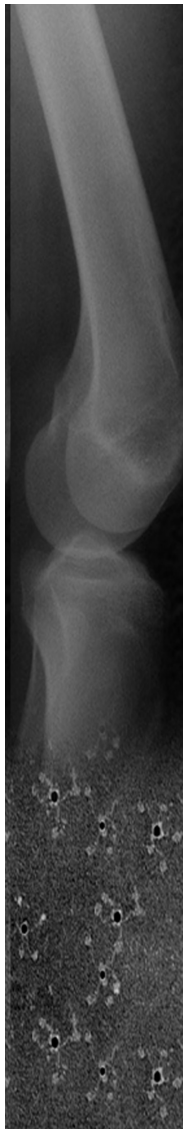


Animal COMP® ELISA

Enzyme Immunoassay

Directions for Use

AnaMar Medical

INTENDED USE

Animal COMP ELISA from AnaMar provides a method for the determination of Cartilage Oligomeric Matrix Protein (COMP) in Rat, Mouse, Sheep, Bovine, Pig and Goat serum.

SUMMARY AND EXPLANATION OF THE TEST

COMP is a protein which is released in the blood when cartilage is destroyed and can be used prognostically for cartilage destruction in inflammatory joint diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA). A quantitative relation between COMP concentration in serum and the degree of cartilage destruction has been shown.

PRINCIPLE OF THE PROCEDURE

In the competitive COMP ELISA, bovine COMP is used to coat the microtiterplates and serum from rats as calibrators. A polyclonal antisera directed against COMP from rats is used as the primary antibody and is incubated together with samples and calibrators directly in the microtiterplate. After the wash a secondary antibody is added to the well. The plate is incubated, developed and read at 450 nm. The response is inversely proportional to the concentration of Animal COMP in the sample.

REAGENTS

Each Animal COMP ELISA kit contains reagents for 96 wells, which is sufficient for one calibrator curve and 42 samples in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2-8 °C. However, each component is stable until the date stated on the respective label.

COMP Coated Plate (COMP of Bovine origin)	1 plate 96 wells 8-well strips	Ready for use *Store at 2-8 °C until expiry date.* *For unused microtitration strips, reseal the bag and store at 2-8 °C for two months.*
Calibrators (Rat origin) Concentration indicated on vial label	5 vials 0.5 ml	Ready for use *Store at 2-8 °C until expiry date.*
Sample Buffer	1 vial 7.5 ml	Ready for use *Store at 2-8 °C until expiry date.*
Polyclonal Antibody	1 vial 7 ml	Ready for use *Store at 2-8 °C until expiry date.*

Enzyme Conjugate 11x Peroxidase conjugated antibody (Donkey origin)	1 vial 1.2 ml	Concentrate *Store at 2-8 °C until expiry date.* Preparation, see table. **Do not freeze!**
Conjugate Buffer	1 vial 12 ml	Ready for use *Store at 2-8 °C until expiry date.*
Stop Solution 0.5 M H_2SO_4	1 vial 7 ml	Ready for use *Store at 2-8 °C until expiry date.*
Washing Tablet	2 Tablets	Dissolve 1 tablet in 500 ml distilled water
Enzyme Substrate (TMB)	1 vial 12 ml	Ready for use *Store at 2-8 °C until expiry date.* **Light sensitive!**

PREPARATION AND HANDLING OF SAMPLES AND ENZYME CONJUGATE

Samples

Blood should be collected by venipuncture, allowed to clot, and the serum separated by centrifugation. Specimens can be stored for 2-4 weeks at 2-8 °C. For longer periods store specimens at -20 °C.

Preparation of samples

Samples should be diluted 1/10 in sample buffer.

Preparation of Enzyme Conjugate

Prepare the enzyme conjugate by dilution of enzyme conjugate 11X, (1+10) in conjugate buffer.

Note: Conjugate volumes are preferably adjusted by diluting all at once, or prepare the needed volume according to the table below. Mix gently.
Store diluted conjugate at 2-8 °C for up to four weeks.

Number of strips	Enzyme Conjugate 11X	Conjugate Buffer
4 strips	350 µl	3.5 ml
6 strips	500 µl	5.0 ml
12 strips	1 vial	1 vial

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use. Not for internal or external use in humans or animals.
- The contents of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- This kit contains no material of human origin.

For the handling of blood, (serum), we recommend that precautions should be observed.

Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/ national guidelines on laboratory safety procedures.

The Stop Solution consists of diluted sulfuric acid solution. Avoid exposure to bases, metals, or other compounds that may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns avoid contact with skin and eyes.

PROCEDURE

Parameters of the Procedure

Volumes per well:	
Sample	50 µl
Calibrators	50 µl
Polyclonal Antibody	50 µl
Conjugate	100 µl
Enzyme Substrate	100 µl
Stop Solution	50 µl

Incubation Time and Temperature:

1st incubation	120 min. on a plate shaker at room temperature, 20-28 °C, (RT).
2nd incubation	60 min. on a plate shaker at RT
3rd incubation	15 min. at RT

Materials Required but not Provided

50 µl, 100 µl micropipette with disposable tips
50 µl, 100 µl repeating pipettes
(multi channel micropipette with disposable tips)
1000 ml beaker

Distilled water

EIA plate reader with 450 nm filter

Plate shaker

Wash device for microtitration plates

Test Procedure

Prepare a calibrator curve for each assay run. All reagents and samples must be brought to room temperature before use. Perform each determination in duplicate for calibrators and unknowns.

Prepare wash buffer by disolving one Washing Tablet in 500 ml distilled water, sufficient for 96 wells.

Add to microtiter wells

1.	Calibrators	50 µl
2.	Diluted samples	50 µl
3.	Polyclonal antibody to all wells	50 µl
4.	Incubate for 2 hours at room temperature on a shaker.	
5.	Wash 6 times with automatic washer, or: Aspirate the reaction volume. Add 350 µl Washing solution to each well. Aspirate completely. Repeat 5 times. After the final wash, invert and tap the plate firmly against absorbent paper.	
6.	Add 100 µl Conjugate to each well.	
7.	Incubate for 1 hours at room temperature at slow shaking/rotation.	
8.	Wash 6 times with automatic washer, or: Aspirate the reaction volume. Add 350 µl Washing solution to each well. Aspirate completely. Repeat 5 times. After the final wash, invert and tap the plate firmly against absorbent paper.	
9.	Add 100 µl Enzyme substrate to each well	
10.	Incubate for 15 minutes at room temperature.	
11.	Add 50 µl Stop solution. Put the plate on the shaker for approximately 5 seconds to ensure mixing of substrate and stop solution. (omit if reading device has shaker function).	
12.	Measure absorbance at 450 nm and evaluate.	

Example of Worksheet

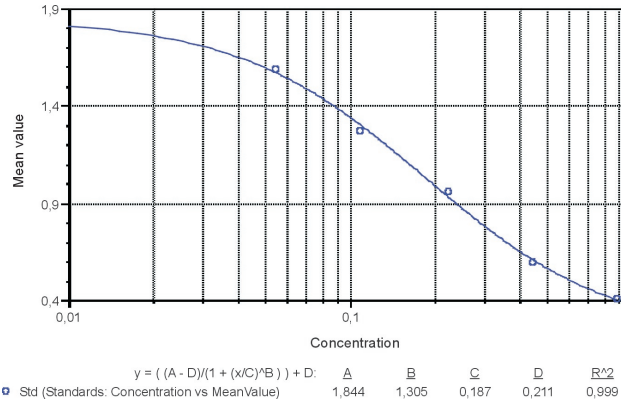
Wells	Identity
1A-B	Sample buffer, (blank)
1C-D	Calibrators 0.9 U/L*
1E-F	Calibrators 0.45 U/L*
1G-H	Calibrators 0.22 U/L*
2A-B	Calibrators 0.11U/L*
2C-D	Calibrators 0.055 U/L*
2E-F	Unknown 1

* Concentration indicated on vial label.

METHOD DATA

Calibrator curve

A typical calibrator curve is shown below. Do not use this curve to determine actual assay reults.



CALCULATION OF RESULTS

Manual calculation

1. Plot the absorbance values obtained for the calibrators against the COMP concentration on a lin-log paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.
3. Multiply the results by the dilution factor (x10).

Computerized calculation

Computerized data reduction of absorbance for the calibrators versus the concentration using a 4 parameter (or cubic) regression may be performed to obtain the concentration of COMP.

PERFORMANCE CHARACTERISTICS

Detection limit

The detection limit is <0.2 U/L.

Parallelism

Serum samples were diluted 5, 10, and 20 times using the sample diluent. The observed Obtained/Expected values for 1/5 to 1/20 were in the range 94-114%.

Precision

Each sample was analysed in 4-replicates on six different occasions.

Sample	Mean value	Coefficient of variation		
	U/L	within assay %	between assay %	total assay %
1	2.8	9.8	6.0	11.5
2	3.5	9.9	5.9	11.5
3	1.7	8.5	7.6	11.5

WARRANTY

The performance data presented here were obtained using the recommended procedure indicated. Any change or modification in the procedure not recommended by AnaMar Medical may affect the results, in which event AnaMar Medical disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

In such an event, AnaMar Medical and its authorized distributors shall not be liable for damages indirect or consequential.

EXPLANATION OF SYMBOLS USED ON LABELS

	Reagents for 96 determinations
	Expiry date
	Store at 2-8°C
	Lot no
	Manufactured by

REFERENCES

1. Larsson E, Erlandsson Harris H, Larsson A, Mansson B, Saxne T, Klareskog L. "Corticosteroid treatment of experimental arthritis retards cartilage destruction as determined by histology and serum COMP." Rheumatology (Oxford). 2004 Apr;43(4): 428-34.

2. Larsson E, Erlandsson Harris H, Lorentzen JC, Larsson A, Mansson B, Klareskog L, Saxne T. "Serum concentrations of cartilage oligomeric matrix protein, fibrinogen and hyaluronan distinguish inflammation and cartilage destruction in experimental arthritis in rats." Rheumatology (Oxford). 2002 Sep;41(9):996-1000.

3. Joosten LA, Lubberts E, Helsen MM, Saxne T, Coenen-de Roo CJ, Heinegard D, van den Berg WB. "Protection against cartilage and bone destruction by systemic interleukin-4 treatment in established murine type II collagen-induced arthritis." Arthritis Res. 1999;1(1):81-91.

4. Joosten LA, Helsen MM, Saxne T, van De Loo FA, Heinegard D, van Den Berg WB. "IL-1 alpha beta blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-alpha blockade only ameliorates joint inflammation." J Immunol. 1999 Nov 1;163(9):5049-55.

5. Joosten LA, Helsen MM, Saxne T, Heinegard D, van de Putte LB, van den Berg WB. "Synergistic protection against cartilage destruction by low dose prednisolone and interleukin-10 in established murine collagen arthritis." Inflamm Res. 1999 Jan;48(1): 48-55.

6. Larsson E, Mussener A, Heinegard D, Klareskog L, Saxne T. "Increased serum levels of cartilage oligomeric matrix protein and bone sialoprotein in rats with collagen arthritis." Br J Rheumatol. 1997 Dec;36(12):1258-61.

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