

Animal COMP ELISA

Catalog Number A-COMP.96

For the quantitative determination of COMP (Cartilage Oligomeric Matrix Protein) in rat, mouse, sheep, bovine, and canine serum samples.

For research use only.

This product insert must be read in its entirety before using this product.

SUMMARY AND EXPLANATION

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 ASSAY

In the competitive COMP ELISA, bovine COMP is used to coat the microtiter plates 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 microtiter plate. 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.

KIT COMPONENTS

Microtiter plate - The plate contains 12 x 8 strips coated with bovine anti-COMP. The strips are ready to use.

Sample buffer - 7.5 mL of buffer with additives.

Wash Tablet - 2 tablets to be dissolved in distilled water.

Calibrators - 5 vials (0.5 mL/vial) of rat COMP in buffer. Concentration indicated on vial label.

Polyclonal Antibody - 7 mL of anti-COMP antibody.

Enzyme Conjugate 11X - 1.2 mL of a concentrated solution of peroxidase conjugated donkey anti-COMP antibody.

Conjugate Buffer - 12 mL of buffer with preservatives.

Enzyme Substrate - 2 vials (6 mL/vial) of a TMB solution ready to use. Protect from light.

Stop Solution - 7 mL 0.5 M H₂SO₄. *Warning: Stop solution contains 0.25 M sulphuric acid. Wear eye, hand, face, and clothing protection when using this material!*

STORAGE

Unopened kit	Store at 2 - 4° C. Do not use past the kit expiration date.	
Opened/Reconstituted Reagents	Calibrators	Store at 2 - 8° C until expiration date.
	Sample Buffer	
	Conjugate Buffer	
	Polyclonal Antibody	
	Enzyme Conjugate 11X	
	Substrate	
	Stop Solution	
	Diluted Enzyme Conjugate	Store at 2 - 8° C for up to 4 weeks.
Microtiter Wells	Return unused wells to the foil pouch containing the desiccant and seal. Store at 2 - 8° C for 2 months.	

SUPPLIES REQUIRED BUT NOT PROVIDED

- Pipettes or pipetting equipment with disposable polypropylene tips (50 µL, 100 µL)
- Glass measuring cylinders
- Distilled or deionised water
- Horizontal orbital microplate reader
- Squirt bottle or automated microplate washer
- Microplate reader capable of measuring at 450 nm

PRECAUTIONS

Warning: Stop Solution consists of diluted sulfuric acid. Wear eye, hand, face, and clothing protection when using these materials. Avoid contact with skin and eyes. In case of contact wash immediately with water. All chemicals should be considered as being potentially hazardous. All chemicals should be considered as being potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice.

- 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.

CRITICAL PARAMETERS

- Allow samples and all reagents to equilibrate to room temperature (20 - 30 °C) prior to performing the assay. This is especially a prerequisite for the TMB Substrate!
- It is absolutely important that all wells are washed thoroughly and uniformly. When washing is done by hand, use a squeeze bottle and ensure that all wells are completely filled and emptied at each step.
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.
- A separate standard curve must be run on each plate.
- Mix all reagents thoroughly prior to use, but avoid foaming!
- Keep the wells sealed with the foil except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for all validated sample types, as designated in this manual. Other sample types may be tested and validated by the user.

SAMPLE COLLECTION AND STORAGE

Serum - Blood should be collected by venipuncture, allowed to clot, and the serum separated by centrifugation. Remove serum and assay immediately. Samples may be stored at 2 - 8 °C for up to 4 weeks or at -20 °C for longer storage. Avoid freeze-thaw cycles.

SAMPLE PREPARATION

Samples require at least a 10-fold dilution with Sample Buffer.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Enzyme Conjugate - Dilute the Enzyme Conjugate 11X 10-fold with Conjugate Buffer by adding 1.2 mL of Enzyme Conjugate 11X to 12 mL of Conjugate Buffer. Mix gently. Diluted Conjugate may be stored at 2 - 8 °C for up to 4 weeks. If diluted conjugate is not expected to be used within 4 weeks, then make only the required amount for the assay.

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

Washing Tablet - Dissolve 1 tablet in 500 mL of distilled water and mix thoroughly.

ASSAY PROTOCOL

Read the entire protocol before beginning the assay. It is recommended that all standards and samples be assayed in duplicate. *Note: Reagents and samples may require specific handling temperatures and need preparation prior to the assay. See the Reagent and Sample Preparation sections before proceeding.*

1. Prepare all reagents and samples as described in the previous sections.
2. Remove any excess microtiter strips from the plate frame and return them to the foil pouch containing the desiccant pack.

Calibrator/Sample/Antibody Incubation

3. Pipette 50 μ L of calibrators or diluted sample in duplicate in the wells using a clean pipette tip for each calibrator or sample.
4. Pipette 50 μ L of polyclonal antibody to the wells. Incubate on a shaker at room temperature for 2 hours.

Wash

5. Aspirate and wash the wells 6 times with Wash Buffer. Take care that all wells are completely filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.

Conjugate Incubation

6. Add 100 μ L of Conjugate into each well. Incubate on a slow shaker at room temperature for 1 hour.

Wash

7. Aspirate and wash the wells 6 times with Wash Buffer. Take care that all wells are completely filled and emptied at each wash. Blot the plate on paper towels to remove residual fluid from the plate.

Substrate Incubation

8. Add 100 μ L of Enzyme Substrate to each well. Incubate at room temperature for 15 minutes.

Stop Reaction

9. Stop the reaction by adding 50 μ L of Stop Solution to each well. Place on shaker for 5 seconds to ensure complete mixing or use shaker function on reading device.
10. Read the plate at 450 nm.

SUMMARY

Prepare reagents and samples as previously described.



Pipette 50 μ L calibrator or diluted sample in duplicate into the wells.



Pipette 50 μ L polyclonal antibody into the wells. Incubate 2 hrs. at RT on a shaker.



Aspirate and wash 6 times.



Add 100 μ L of Conjugate to each well. Incubate 1 hour at RT on a shaker.



Aspirate and wash 6 times.



Add 100 μ L of Enzyme Substrate to each well. Incubate 15 minutes at RT.



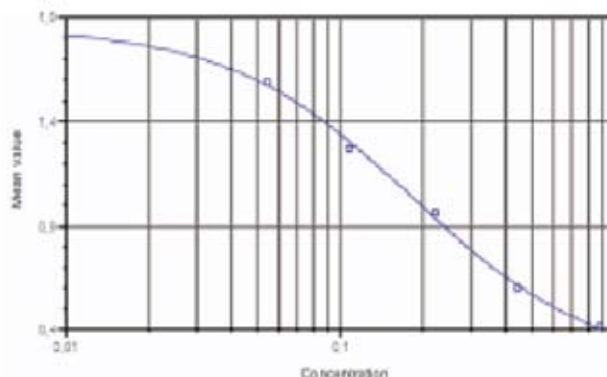
Add 50 μ L of Stop Solution to each well. Read at 450 nm

CALCULATION OF RESULTS

The calibrator curve is provided for demonstration only. A calibrator curve should be generated with each set of samples assayed.

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

Sensitivity

The sensitivity, defined as two standard deviations above the concentration mean of the calculated concentrations of several blank replicates was determined. The sensitivity was determined as <0.2 U/L.

Reproducibility

Intra-assay Precision (Precision within an assay) - The intra-assay precision was measured by assaying three control samples 4 times on one plate.

Inter-assay Precision (Precision between assays) - The inter-assay precision was assessed by repeated measurements of three control samples in 6 successive assays.

Control	Intra-assay Precision			Inter-assay Precision			Total Assay Precision		
	1	2	3	1	2	3	1	2	3
Mean (U/L)	2.8	3.5	1.7	2.8	3.5	1.7	2.8	3.5	1.7
CV (%)	9.8	9.9	8.5	6.0	5.9	7.6	11.5	11.5	11.5

PARALLELISM

Serum samples were diluted 5, 10, and 20 times using Sample Diluent. The observed Obtained/Expected values for 1/5 to 1/20 ranged from 94 - 114%.

■ USA:

MD Biosciences, Inc.
1000 Westgate Dr, Suite 162
St. Paul, MN 55114
toll-free: 1-888-USMDBIO
tel: (651) 641-1770
fax: (651) 641-1773

▲ Europe:

div. of Morwell Diagnostics GmbH
Gewerbestrasse 9
8132 Egg b. Zürich, Switzerland
tel: (+41-1) 986 2628
fax: (+41-1) 986 2630

■ Israel:

Sapir Street 3
Weizmann Science Park
Nes-Ziona, 74140 Israel
toll free: 1 800 200 MDB
tel: 972 (0)8 9396884
fax: 972 (0)8 9396885