PRODUCT INFORMATION & ELISA MANUAL

COMP/Thrombospondin-5 Antibody Pair [Biotin]

NBP2-79472

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked Immunosorbent Assay for quantitative detection of Human COMP/Thrombospondin-5.

For research use only.
Not for diagnostic or therapeutic procedures.

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BACKGROUND

Cartilage Oligomeric Matrix Protein (COMP), also referred to as thrombospondin-5, is a secreted noncollagenous extracellular matrix (ECM) protein and belongs to the subgroup B of the thrombospondin protein family. The native glycoprotein incorporates five identical subunits, each with the molecular weight of 87 KDa consists of four epidermal growth factor (EGF)-like domains, eight so-called thrombospondin type 3 (T3) repeats (calcium-binding domain) and a carboxyl-terminal globular domain. A coiled-coil domain at the amino terminus mediates the pentamerization resulting in a bouquet-shaped subunit arrangement via disulfide-bonds. COMP is a component of cartilage, synovium, ligament, and tendon, and binds to other ECM proteins such as collagen I, II and IX with high affinities depending on the divalent cations Zn^{2+} or Ni^{2+} . COMP is suggested to play a role in cell growth and development, and recent studies have revealed the possible mechanism that it protects cells against death by elevating members of the IAP (inhibitor of apoptosis protein) family of survival proteins. COMP mutations cause dominantly inherited chondrodysplasias Pseudoachondroplasia (PSACH) and multiple epiphyseal dysplasia (MED) characterized by short stature and early-onset osteoarthrosi, and up-regulated expression of COMP are observed in rheumatoid arthritis and certain carcinomas

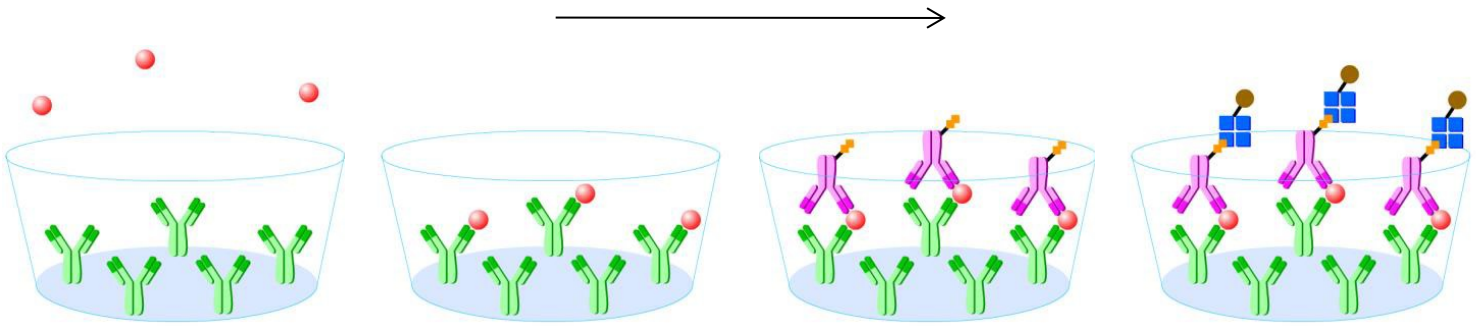
PRINCIPLE OF THE TEST

The Novus Biologicals COMP/Thrombospondin-5 Antibody Pair [Biotin] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for COMP/Thrombospondin-5 coated on a 96-well plate. Standards and samples are added to the wells, and any COMP/Thrombospondin-5 present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti- COMP/Thrombospondin-5 polyclonal antibody is then added, producing an antibody-antigen-antibody “sandwich”. To produces color in proportion to the amount of COMP/Thrombospondin-5 present in the sample strepavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450 nm.

INTENDED USE

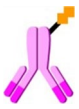
- The human COMP/Thrombospondin-5 Antibody Pair [Biotin] is for the quantitative determination of human COMP/Thrombospondin-5.
- This COMP/Thrombospondin-5 Antibody Pair [Biotin] contains the basic components required forthe development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



Capture Antibody

Analyte



Biotinylated Detection Antibody

HRP-conjugated Streptavidin

This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody - 0.5 mg/mL of mouse anti-COMP/Thrombospondin-5 monoclonal antibody. Dilute to a working concentration of 2 µg/mL in CBS before coating.

Detection Antibody - Each vial contains 120 µg biotinylated rabbit anti-COMP/Thrombospondin-5 polyclonal antibody. Reconstitute with sterile 1 mL distilled water. Dilute to a working concentration of 2 µg/mL in detection antibody dilution buffer before use

Standard - Each vial contains 60 ng of recombinant COMP/Thrombospondin-5. Reconstitute with 1 mL detection antibody dilution buffer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 2500 pg/mL is recommended

Streptavidin-HRP - 50 µL of streptavidin conjugated to horseradish-peroxidase. 1:2000 Dilution in detection antibody dilution buffer before use

SOLUTIONS REQUIRED

CBS - 0.05M Na_2CO_3 , 0.05M $NaHCO_3$, pH 9.6, 0.2 µm filtered

TBS - 25mM Tris, adjust pH to 7.4 by HCl

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered.

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10 mg/ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na_2HPO_4 and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution buffer and then add 80 µl 0.75% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Standard: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -20℃ to -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

Streptavidin-HRP: Store at 4℃ and protect it from prolonged exposure to light. **DO NOT FREEZE!** It is stable for up to 6 months from date of receipt.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

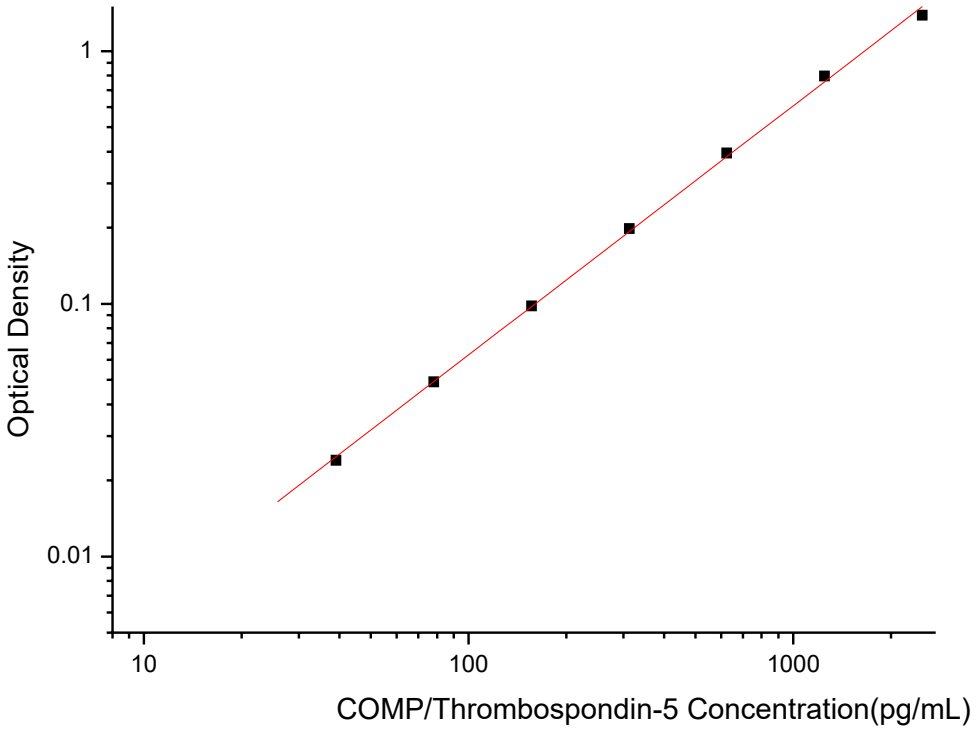
1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 µL of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.
8. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- 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.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
39.0625	0.024
78.125	0.049
156.25	0.098
312.5	0.198
625	0.395
1250	0.796
2500	1.384

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of human COMP/Thrombospondin-5 was determined to be approximately **39.1 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Human COMP/Thrombospondin-5 Antibody Pair [Biotin]

Notes