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Antibodies | ELISA kits | Proteins

Human COMP Sandwich ELISA Kit Datasheet

For the quantitative detection of human COMP concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00105
Product Name	Human COMP Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25 - 2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	1311
SwissProt	P49747

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection Antibody, biotinylated (100×) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	2 bottles	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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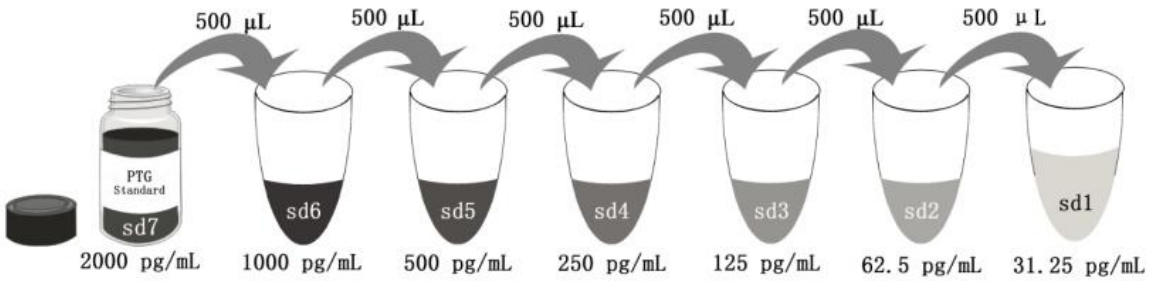
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ef	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00105 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The COMP ELISA kit is to be used to detect and quantify protein levels of endogenous COMP. The assay recognizes human COMP. An antibody specific for COMP has been pre-coated onto the microwells. The COMP protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human COMP is added to detect the captured human COMP protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

COMP is a secreted pentameric glycoprotein built from modular units and belongs to the thrombospondin protein family. COMP is expressed by hypertrophic chondrocytes and osteoblasts during endochondral ossification, and its expression is increased during fracture healing. COMP is involved in the assembly of collagen fibrils and other structural matrix components. COMP interacts with a number of cartilage ECM proteins, including fibronectin, collagen I, II IX, XII, and XIV, matrilins, as well as proteoglycans such as aggrecan and others.

Sample Preparation

Different samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:160 or 1:320 before assay, 1:2 or 1:4 dilution is recommended for cell culture supernatants.

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Safety Notes

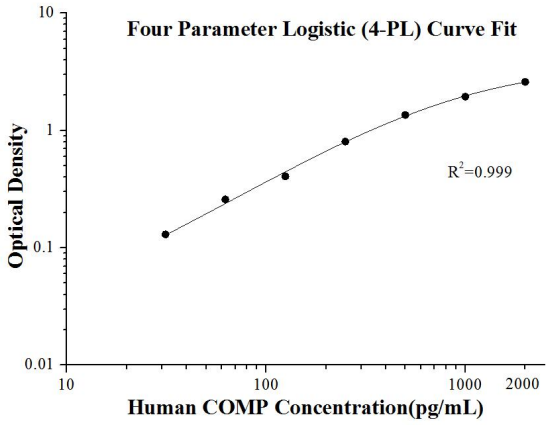
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.036 0.037	0.037	-
31.25	0.16 0.172	0.166	0.13
62.5	0.29 0.298	0.294	0.258
125	0.383 0.501	0.442	0.406
250	0.842 0.839	0.841	0.804
500	1.398 1.386	1.392	1.356
1000	2.033 1.922	1.978	1.941
2000	2.631 2.629	2.63	2.594

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	818.1	70.1	8.6
2	20	194.2	18.3	9.4
3	20	51.5	4.6	9.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	874.3	81.0	9.3
2	24	206.0	20.5	9.9
3	24	44.2	4.4	9.9

Recovery

The recovery of COMP spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:160	100	76-127
	1:320	107	87-121
Cell culture supernatants	1:2	105	73-122
	1:4	110	101-122

Sample Values

Serum and plasma samples from healthy volunteers (human) were evaluated for COMP in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=24)	94.3	14.3-401.7
Human plasma (n=16)	89.5	34.3-192.8

Sensitivity

The minimum detectable dose of human COMP is 1.1 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, cell culture supernatants samples were spiked with high concentrations of human COMP in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Serum were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.(The serum samples were initially diluted 1:80)

		Human serum	Cell culture supernatants
1:2	Average% of Expected	100	117
	Range (%)	-	115-119
1:4	Average% of Expected	92	121
	Range (%)	83-104	111-130
1:8	Average% of Expected	94	98
	Range (%)	84-106	97-100
1:16	Average% of Expected	94	86
	Range (%)	88-107	85-88

References

1. Di Cesare PE. et al. (2000). J Orthop Res. 18(5):713-20.
2. Acharya C. et al. (2014). Matrix Biol. 37:102-11.
3. Thur J. et al. (2001). J Biol Chem. 276(9):6083-92.
4. Hedbom E. et al.(1992). J Biol Chem. 267(9):6132-6.

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