Anti CCP GENLISA™ ELISA

REF : KBD281

Ver 2.2

IVD

Enzyme Immunoassay for the Quantitative Determination of Anti CCP in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBD281 **Σ 96 tests**

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Introduction:

The CCP (cyclic citrullinated peptide) antibody is an autoantibody against citrullinated proteins (ACPA). They are present in the majority of patients with rheumatoid arthritis. Rheumatoid arthritis (RA) is the most common inflammatory autoimmune disorder, causing progressive joint destruction as a result of chronic synovitis. Clinically, cyclic citrullinated peptides (CCP) are frequently used to detect these antibodies in patient serum or plasma (then referred to as anti–citrullinated peptide antibodies.

Intended Use:

The Anti CCP GENLISA™ ELISA is intended for the quantitative determination of IgG class of autoantibodies specific to Anti CCP in human serum and plasma.

Principle:

The Anti CCP GENLISA™ ELISA is an indirect enzyme linked immnunosorbent assay for quantitative determination of IgG antibody present in human serum and plasma. The Microtiter wells are pre-coated with Streptavidin. Biotinylated CCP Peptide, standards, serum samples and Controls are pipetted into Streptavidin-coated wells. After incubation and washing, Anti-Human IgG HRP Conjugate is added. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of the Anti CCP present in the sample. Color development is then stopped by adding stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Streptavidin coated Microtiter Plate (8x12 wells) - 1 no
2. CCP Standards (1.0 ml/vial) - 0, 10, 25, 75, 125, 250 RU/ml
3. Sample diluent – 2 X 50 ml
4. Low Control Serum – 1 ml
5. High Control Serum – 1 ml
6. Biotinylated CCP Peptide – 3 ml
7. Anti-Human IgG:HRP Conjugate (concentrated)(20X) - 1 ml
8. HRP Conjugate Diluent - 12 ml
9. (10X) Wash Buffer – 2 X 50 ml
10. TMB Substrate - 12 ml
11. Stop Solution - 12 ml
12. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 10 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Sample Dilution:
To make 1:101 dilutions, dilute 10 ul sample with 1000ul sample diluent. Mix well.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature, 18-25°C) prior to using the assay.
2. **Anti-Human IgG:HRP Conjugate working solution –** To prepare the required amount of conjugate, dilute the concentrated conjugate with the HRP conjugate diluent in a ratio of 1:20. Mix it well. For example, to prepare 1 ml of ready to use Conjugate working solution, mix 50ul of concentrated **Anti-Human IgG:HRP Conjugate** with 950ul of **HRP conjugate diluent**.
3. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 50ml of Wash Buffer (10X) to 450ml of DI water. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **25 ul** of **Biotinylated CCP Peptide** to wells in which you will add the controls, samples, and standards.
3. Add **100 ul Standard, High Control, Low Control and Diluted Sample** in appropriate wells.
4. Cover the plate and incubate for 60 minutes at room temperature (22-28ºC).
5. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from outside the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6. Add **100 ul** of **Anti-Human IgG:HRP Conjugate working solution** to each well.
7. Cover the plate and incubate for 30 minutes at room temperature (22-28ºC).
8. Repeat the Wash Step as mentioned in step 4.
9. Add **100 ul** of **TMB Substrate** into each well.
10. Incubate at room temperature for 15 minutes in dark.
11. Add **100 ul** of **Stop Solution**. Read result with an ELISA reader at 450 nm.

Interpretation of Results:

Using graph paper, plot the mean OD value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Anti CCP concentrations, find the unknown's 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 Anti CCP Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit, 4-PL or a polynomial curve (2nd order) is best recommended for automated results.

Validity of the controls:

The test is valid if the below condition is met:

Low Control Serum: 4.0 – 16 RU/ml
High Control Serum: 65 – 165 RU/ml

Expected Values:

It is important for each laboratory to establish the normal range limits. The following reference range from healthy persons should be considered as a guideline only. Anti-CCP IgG was measured in healthy persons. The mean value obtained was 3.38 RU/ml (95% CI: 2.87 – 3.88) with a standard deviation of 3.76 RU/ml. In this study, 98% of subjects had an Anti-CCP IgG value of 11.0RU/ml. The cut-off has been calculated based on mean divided by 3SD as 15RU/ml.

Performance Characteristics:

1. Comparative Study:

The Anti-CCP IgG GENLISA™ kit was compared with the commercially available Anti-CCP IgG Elisa kit.

2. Minimum Detection Limit:

The LOD of Anti-CCP IgG by this assay is estimated to be **0.6 RU/ml**.

3. Precision:

Intra-Assay precision:

Within assay variability is shown below:

No.	No. of Tests Performed	Means RU/ml	SD RU/ml	CV%
1	20	2.6	0.19	7.3
2	20	50.3	4.5	8.9
3	20	169	8.2	4.9

Inter-Assay precision:

Between assays variability is shown below:

No.	No. of Tests Performed	Means RU/ml	SD RU/ml	CV%
1	20	2.5	0.26	10.4
2	20	47.2	5.3	11.2
3	20	167	9.0	5.4

*Each test has been run in duplicate

4. Interference:

To determine the interferences with the kit, the following amounts of hemoglobin, triglyceride and bilirubin were added to three specimens and the neutralizing antibody concentration was measured before and after the involvement of the substances mentioned above the results is shown in the table below:

Interferent Analyte	The concentration of the interferent analyte	The value of the specimen before adding the interferent (RU/ml)	The value of the specimen after adding the interferent (RU/ml)	The change of the results (%)
Hemoglobin	1 mg/ml	3.3 19.6 163	3.3 19.9 162	-1.1 1.5 -1.0
Triglyceride	3000 mg/dl	3.3 19.6 163	3.4 18.9 164	1.1 -3.5 0.67
Bilirubin	20 ng/dl	3.36 19.6 163	3.3 20.1 162	0.29 2.5 -1.0

5. Test Linearity:

To verify test linearity, three different serum samples with known Anti-CCP IgG concentrations were diluted sequentially by sample diluent. Then the Sera were tested by the ELISA. The results and serum recovery were determined considering the dilution factor.

		Recovery (%)			
No.	**Anti-CCP IgG (RU/ml) Undiluted specimen**	**1:2**	**1:4**	**1:8**	**1:16**
1	**248**	**106**	**86.9**	**114**	**96.2**
2	**202**	**103**	**93.7**	**91.7**	**81.7**
3	**148**	**96.6**	**93.6**	**87.4**	**91.1**

6. Test Recovery:

Certain amounts of Anti-CCP IgG were added to 3 different sera with known concentrations of Anti-CCP IgG and then their recovery was determined. The results are shown below:

No.	Anti-CCP IgG level RU/ml	Anti-CCP IgG added RU/ml	Exp. RU/ml	Obs. RU/ml	Rec. (%)
1	201	115	158	143	90.5
2	74.6	115	95.2	104	109
3	8.4	115	62.1	61.5	99.0
1	201	50.6	125	114	91.2
2	74.6	50.6	62.6	59.1	94.4
3	8.4	50.6	29.5	26.8	90.8
1	201	188	194	207	106.7
2	74.6	188	131	120	91.6
3	8.4	188	98.2	91.6	93.3

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.

- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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EC REP **KinesisDx, Lyoner Strasse 14, Frankfurt, Germany**

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

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2	Add **25 ul** of **Biotinylated CCP Peptide** to wells in which you will add samples, controls, and standards.
3	Add **100 ul Standard**, **High Control**, **Low Control** and **Diluted Sample** in appropriate wells.
4	Cover the plate and incubate for 60 minutes at room temperature (22-28ºC).
5	Aspirate and wash plate 5 times with (1x) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6	Add **100 ul** of **Anti-Human IgG:HRP Conjugate** to each well.
7	Cover the plate and incubate for 30 minutes at room temperature (22-28ºC).
8	Repeat the Wash Step as mentioned in step 4.
9	Add **100 ul of TMB Substrate** into each well.
10	Incubate for 15 minutes at room temperature (22-28ºC).
11	Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction.

SYMBOLS KEY

MTP	Microtiter Plate (8x12 wells)
STD	Standards
SAMP DIL	Sample Diluent
LC	Low Control Serum
HC	High Control Serum
BIOTIN CCP	Biotinylated CCP
20X HRP CONJ	Anti-Human- IgG:HRP Conjugate
HRP DIL	HRP Conjugate Diluent
10X WASH BUF	(10X) Wash Buffer
SUB TMB	TMB Substrate
SOLN STOP	Stop Solution
	Consult Instructions for Use
REF	Catalog Number
	Expiration Date
	Storage Temperature