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Instructions for Use

CCP-Ab ELISA

Enzyme immunoassay for the quantitative determination of IgG autoantibodies to cyclic citrullinated peptides (CCP) in human serum or plasma.

REF **RE75791**

Σ **96**

2-8 °C

EU: IVD CE

U.S.: *For research use only.*
Not for use in diagnostic procedures.

I B L I N T E R N A T I O N A L G M B H

Flughafenstrasse 52a
D-22335 Hamburg, Germany

Phone: +49 (0)40-53 28 91-0
Fax: +49 (0)40-53 28 91-11

IBL@IBL-International.com
www.IBL-International.com

1. INTENDED USE

The CCP-Ab ELISA is used for the quantitative determination of IgG antibodies against cyclic citrullinated peptides (CCP) in human sera or plasma.

2. SUMMARY AND EXPLANATION

Rheumatoid Arthritis (RA) is most often diagnosed by the measurement of rheumatoid factors (RF). The rheumatoid factor is an antibody directed against the Fc-region of IgG. It appears mainly as IgM antibody but also as IgA or IgG subgroup.
Rheumatoid factors are present in sera of 70-80 % of patients suffering from rheumatoid arthritis. But it is not specific for RA since it will also be found in healthy persons. The incidence depends on the age: for young people it is 1-4 %, for older persons it may reach up to 25 %.
The advantage of CCP antibodies is a higher sensitivity and specificity for the diagnosis of rheumatoid arthritis in comparison to the rheumatoid factors alone. Anti-CCP is often found at a very early state of the disease and it has a high predictive value for development of the disease.

3. TEST PRINCIPLE

In the first step CCP AAb from the diluted sample (as well as from the calibrators and control) bind to cyclic citrullinated peptides coated on the microtiter plate. After an incubation of 60 minutes at room temperature (RT) unbound components are removed by washing.
In a next step bound antibodies reacts with the added anti-human-IgG horseradish peroxidase (HRP) complex. Excessive conjugate is removed after 30 minutes at RT by another washing step.
HRP converts the colorless substrate TMB added into a blue product. The enzyme reaction is stopped by adding an acid solution after 15 minutes at RT. The color changes from blue to yellow. The absorbance of the resulting product is measured at 450/620 nm within 30 minutes. The obtained OD is direct proportional to the amount of bound antibodies.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. All reagents should be kept at 2 - 8°C before use in the original shipping container.
10. Some of the reagents contain small amounts (< 0.1% w/v) Thimerosal and (1% v/v) Kathon as a preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.
11. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

5. STORAGE AND STABILITY

Allow the sealed microplate to reach room temperature before opening for at least 30 minutes. Unused wells should be stored refrigerated and protected from moisture in the original bag. Carefully resealed it can be used for 8 weeks.
The anti-human IgG-HRP solution is stable up to 8 weeks at 2-8 °C after opening. Avoid exposure of substrate solution to light. The expiry date of each component is reported on its respective label, that one of the complete kit on the box label. Upon receipt, all components of the CCP-Ab ELISA have to be kept at 2-8 °C, preferably in the original kit box.

6. SPECIMEN COLLECTION AND STORAGE

Serum, Plasma

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8 °C	-20 °C (Aliquots)	Keep away from heat or direct sun light.
Stability:	< 3 d	> 3 d	Avoid repeated freeze-thaw cycles.

7. MATERIALS SUPPLIED

⚠ The reagents provided with this kit are sufficient for up to 96 determinations. This is sufficient for the analysis of 42 unknown samples as well as for calibrators and control serum assayed in duplicates.

Quantity	Symbol	Component
1 x 12 x 8	MTP	**Microtiter Plate** Break apart strips. Coated with synthetic peptides with citrulline residues.
1 x 15 mL	ENZCONJ	**Enzyme Conjugate** Ready to use Contains: Anti human IgG (sheep) Horseradish-peroxidase (HRP) complex
5 x 1 mL	CAL	**Calibrators 1-5** Ready to use Exact concentrations see vial labels or QC certficate.
1 x 1 mL	CONTROL+	**Positive Control** Exact concentrations see vial labels or QC certficate.
1 x 1 mL	CONTROL-	**Negative Control** Exact concentrations see vial labels or QC certficate.
1 x 100 mL	SAMPLEDIL	**Sample Diluent** Ready to use.
1 x 100 mL	WASHBUF CONC	**Wash Buffer,** Concentrate (10 x) Sufficient for 1000 mL.
1 x 15 mL	TMB SUBS	**TMB Substrate** Ready to use. Contains: 3,3´,5,5´-Tetramethylbenzidin.
1 x 15 mL	TMB STOP	**TMB Stop Solution** Ready to use. Contains: 0.25 M sulfuric acid.

The CCP-Ab ELISA is artificially calibrated and concentrations of anti-CCP are therefore expressed in U/mL. Those units show a constant factor (1:12) to the WHO Reference standard W1066 for rheumatoid arthritis.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision pipettes 5 - 1000 µL
2. Multi-channel pipette with disposable pipette tips
3. 8 channel wash comb or microplate washer
4. Micro plate reader with optical filters for 450 nm and 620 or 690 nm
5. Graduated cylinders
6. Distilled or de-ionized water
7. Absorbent paper or paper towel
8. Tubes (2 mL) for sample dilution
9. Foil

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
8. Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

10. PRE-TEST SETUP INSTRUCTIONS

10.1. Preparation of lyophilized or concentrated Components

Dilute / dissolve	Component		Diluent	Relation	Remarks	Storage	Stability
50 mL	WASHBUF CONC	with 450 mL	bidist. water	1:9	Warm up at 37 °C to dissolve crystals, if necessary.	2-8 °C	up to 30 days

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

10.2. Dilution of Samples

Sample	to be diluted	with	Relation	Remarks
Serum	generally	SAMPLEDIL	1:100	e.g. 5 µL sample + 500 µL SAMPLEDIL

11. TEST PROCEDURE

1.	Pipette **100 µL** of each **Standard, Control** and **diluted sample** into the respective wells of the Microtiter Plate.
2.	Cover plate with adhesive foil. **Incubate 60 min** at **RT (18-25 °C).**
3.	Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash plate **3 x** with **300 µL** of **diluted Wash Buffer** with 5 seconds soaking time each.
4.	Pipette **100 µL** of **Enzyme Conjugate** into each well.
5.	Cover plate with new adhesive foil. **Incubate 30 min** at **RT (18-25 °C).**
6.	Aspirate or "flick out" by striking the wells sharply onto absorbent paper to remove any residual droplets. Wash plate **3 x** with **300 µL** of **diluted Wash Buffer** with 5 seconds soaking time each.
7.	For adding of Substrate and Stop Solution use, if available, an 8-channel Micropipettor. Pipetting should be carried out in the same time intervals for Substrate and Stop Solution. Use positive displacement and avoid formation of air bubbles.
8.	Pipette **100 µL** of **TMB Substrate Solution** into each well and shake shortly.
9.	**Incubate 15 min** at **RT (18-25 °C)** in the dark.
10.	Stop the substrate reaction by adding **100 µL** of **TMB Stop Solution** into each well. Briefly mix contents by gently shaking the plate.
11.	**Measure** optical density with a photometer at **450 nm** versus **620 or 690 nm** within **30 min** after pipetting of the **Stop Solution.**

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the vial labels. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

Quantitative Evaluation

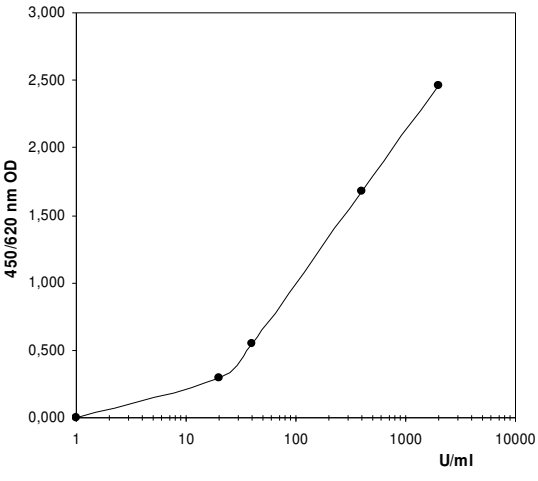
The standard curve is established by plotting the mean OD-values of the calibrators 1 - 5 on the ordinate, y-axis, versus their respective CCP-Abconcentrations on the abscissa, x-axis (log scale).
The CCP-Ab concentrations of the controls and the unknown diluted samples are directly read off in U/mL from the measured OD_{450} values.
There is no further correction for the dilution necessary.
CCP-Ab ELISA may be used also with Computer Assisted Analysis with software able to use spline smoothing fitting. We recommend 4 parameter fit.

Typical Calibration Curve

(Example. Do not use for calculation!!)

Calibrator	OD (a)	OD (b)	OD (mean)	U/mL
1	0.037	0.043	0.040	1
2	0.304	0.285	0.295	20
3	0.514	0.551	0.533	40
4	1.771	1.589	1.680	400
5	2.631	2.284	2.458	2000
Patient Sample 1	1.024	1.019	1.022	103

Specimens with an OD higher than Standard 5 should be diluted further by the sample diluent and the concentration of CCP antibodies should be calculated by the applied dilution factor.



14. INTERPRETATION OF RESULTS

CCP-Ab ELISA	
Range	**Interpretation**
< 30 U/mL	negative
≥ 30 U/mL	positive

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-CCP antibodies levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide only a guide.

15. LIMITATIONS

Healthy individuals should be tested negative by using the CCP-Ab ELISA. However, CCP autoantibodies may also be present in apparently healthy persons.

16. PERFORMANCE

Linearity
On the basic of the heterogeneous nature of the autoantibody population and in view of epitope specificity and affinity of the autoantibodies the theoretical values expected by dilution with anti-CCP free human serum most often correspond with the measured concentrations.

Specificity and sensitivity
The results available show a clinical sensitivity of 79 % at a specificity of 97 % for the diagnosis of rheumatoid arthritis.

Detection limits
The analytical sensitivity (lower detection limit, 0 + 3 SD) was established to be 1.2 U/mL.
The functional sensitivity was measured as 20 % of inter-assay CV at about 2 U/mL.

Intra - and inter-assay variation

Intra-assay		
Sample no.	**Mean Concentration (U/mL)**	**CV (%)**
1	29	7
2	39	3
3	147	7
4	572	5
5	870	6

Inter-assay		
Sample no.	**Mean Concentration (U/mL)**	**CV (%)**
6	2.5	8
7	91	6
8	209	8
9	1140	11
10	1737	9

17. ASSAY SCHEME

Bring all reagents to room temperature. Gently mix all reagents to ensure homogeneity.
Dilute all samples 1 + 100 (v + v) by sample diluent.

Step	Activity	Material	CAL	Control	Diluted patient samples 1, 2 etc.
1	Pipette	Samples	100 µL	100 µL	100 µL
2	Incubate	Plate	**1 hour at RT (18-25 °C)**		
3	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution	3 x 300 µL 5 seconds each	3 x 300 µL 5 seconds each	3 x 300 µL 5 seconds each
4	Pipette	Anti-human IgG HRP	100 µL	100 µL	100 µL
5	Incubate	Plate	**30 min at RT (18-25 °C)**		
6	Aspirate or decant		put sharply onto absorbent tissue		
	Pipette	Washing solution made from	3 x 300 µL 5 seconds each	3 x 300 µL 5 seconds each	3 x 300 µL 5 seconds each
7	Pipette	Substrate	100 µL	100 µL	100 µL
8	Incubate	Plate	**15 min at RT (18-25 °C) in the dark**		
9	Pipette and mix	Stop solution	100 µL	100 µL	100 µL
10	Measure OD		at 450 nm versus 620 nm (or 690 nm) within 30 min		

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.–Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!

Symbols of the kit components see MATERIALS SUPPLIED.
Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.
Voir MATERIEL FOURNI pour les symbôles des composants du kit.
Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.
Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.
Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.
Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

IBL AFFILIATES WORLDWIDE

IBL International GmbH Flughafenstr. 52A, 22335 Hamburg, Germany	Tel.: E-MAIL: WEB:	+ 49 (0) 40 532891 -0 Fax: -11 IBL@IBL-International.com http://www.IBL-International.com
IBL International Corp. 194 Wildcat Road, Toronto, Ontario M3J 2N5, Canada	Tel.: E-MAIL: WEB:	+1 (416) 645 -1703 Fax: -1704 Sales@IBL-International.com http://www.IBL-International.com

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