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DCM149-2
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Anti CP IgG

for routine analysis

Quantitative determination of IgG class antibodies against Citrullinated Peptides (CP) in human serum or plasma

IVD

LOT
See external label

2°C 8°C

Σ = 96 tests

REF DKO149

INTENDED USE

Anti CP IgG is an indirect solid phase enzyme immunometric assay (ELISA) kit designed for the quantitative measurement of IgG class antibodies directed against Citrullinated Peptides (CP) in human serum or plasma.
Anti CP IgG kit is intended for laboratory use only.

1. CLINICAL SIGNIFICANCE

Reumathoid Arthritis (RA) is one of the most common autoimmune diseases (1-2% European population). The most significant clinical symptom is an inflammation of the synovial membranes which causes a painful swelling of the articulations and the ankylosis.
In order to correctly diagnose RA it is necessary to exclude other forms of arthritis: in this diagnostic process, the laboratory plays an important role in the determination of Rheumatoid Factor (RF) antibodies of class IgM, detectable in 60-80% of the patients with RA. The RF antibodies are sensitive but not very specific markers.
On the contrary, anti citrullinated peptides (CP) autoantibodies are a hallmark of RA and are used in diagnostic assays, through the use of citrullinated sequences (such as: filaggrin and filaggrin derived cyclized peptides, citrullinated recombinant vimentin, fibrin, viral citrullinated proteins) as antigens.
Dia.Metra Anti CP IgG assay is based on viral and human citrullinated peptidic sequences that are used as antigens; this combined use allows to detect different subgroups of anti CP antibodies in the same set of patients, leading to a better diagnostic investigation.

2. PRINCIPLE

Anti CP IgG test is based on the binding of antibodies present in calibrators, controls or prediluted patient samples to the syntetic Citrullinated Peptides (CP) coated on the inner surface of the wells. After a 30 minutes incubation the microplate is washed with a wash buffer to remove the non-reactive serum components.
Then an anti-human-IgG horseradish peroxidase conjugated solution recognizes the IgG class antibodies bound to the immobilized antigens. After a 30 minutes incubation, any excess of enzyme conjugate not specifically bound is washed away with the wash buffer.
Finally a chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the color development is stopped by adding the stop solution. The solutions color changes into yellow. The amount of color is directly proportional to the concentration of IgG antibodies present in the original sample.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

CAL0	REF **DCE002/14906-0**
CAL1	REF **DCE002/14907-0**
CAL2	REF **DCE002/14908-0**
CAL3	REF **DCE002/14909-0**
CAL4	REF **DCE002/14910-0**

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

Negative Control	REF **DCE045/14901-0**
Positive Control	REF **DCE045/14902-0**

3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1 M, NaN_3 < 0.1%
REF **DCE053-0**

4. Conjugate (1 vial, 15 mL)
Anti h-IgG conjugated with horseradish peroxidase (HRP), BSA 0.1% REF **DCE002/14902-0**

5. Coated Microplate (1 breakable microplate)
Microplate coated with CP antigens
REF **DCE002/14903-0**

6. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF **DCE004-0**

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF **DCE005-0**

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2M pH 7.4 REF **DCE054-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplates reader (450 nm, 620-630 nm).

Notes

Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of the reagents has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrators and the Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300^R as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
  For this purpose, Dia.Metra supplies a separate decontamination reagent for cleaning needles.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrators (C_0…C_4)

Since no international reference preparation for anti CP antibodies is available, the assay system is calibrated in relative arbitrary units. The Calibrators are ready to use and have the following concentration:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Preparation of the Sample

For determination of anti CP antibodies, human serum or plasma are the preferred sample matrixes.
All serum and plasma samples have to be prediluted with sample diluent 1:100; for example 10 µL of sample may be diluted with 990 µL of sample diluent.
Samples may be stored refrigerated at 2-8°C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20°C. To avoid repeated thawing and freezing the samples should be aliquoted.
The Controls are ready to use.

6.3. Preparation of the Wash Solution

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C. In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagent	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. RESULTS

7.1. Calibration curve

For the Anti CP IgG assay a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the preferred data reduction method. Smoothed-Spline Approximation and log-log coordinates are also suitable.
However we recommend using a Lin-Log curve.
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

8. REFERENCE VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti CP test:

	Anti CP IgG (AU/mL)
Negative	< 10
Positive	> 10

Please pay attention to the fact that the determination of a range of expected values for a "normal" population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti CP.

9. PERFORMANCE AND CHARACTERISTICS

9.1. Specificity

A study performed on 93 samples (45 positive to Rheumatoid Arthritis with positivity to anti CCP antibodies, 48 from healthy blood donors) showed a specificity of 94%.

9.2. Sensitivity

A study performed on 93 samples (45 positive to Rheumatoid Arthritis with positivity to anti CCP antibodies, 48 from healthy blood donors) showed a sensitivity of 76%.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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