



DCM095-7
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Anti dsDNA IgG

for routine analysis

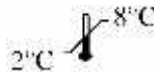
Quantitative determination of IgG class antibodies against dsDNA in human serum or plasma

RUO



LOT

See external label



Σ = 96 tests

REF DKO095

INTENDED USE

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

1. CLINICAL SIGNIFICANCE

Anti dsDNA IgG test is used for initial diagnosis of Systemic Lupus Erythematosus (SLE) and for diagnosis of SLE different diseases. Besides the determination of high titers of antinuclear antibody (ANA), the determination of autoantibodies against dsDNA is one of the ACR criteria (American College of Rheumatology) for the diagnosis of Systemic Lupus Erythematosus (SLE). The determination of the concentration of antibodies can be used to monitor treatment success and predict possible attacks of the disease (SLE)

2. PRINCIPLE

Anti dsDNA IgG test is based on the binding of serum or plasma IgG antibodies on dsDNA coated on the microplates. In the first step the antibodies in calibrators, controls or prediluted patient samples bind into the inner surface of the wells. After an incubation the microplate is washed with a wash buffer for removing non-reactive serum components. In the second step an anti human IgG horseradish peroxidase conjugated solution recognizes the IgG class antibodies bound to the immobilized dsDNA antigens. After an incubation any excess of enzyme conjugate, which is not specifically bound, is washed away with the wash buffer.

Then 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 solution color changes into yellow. The amount of color is directly proportional to the concentration of the Anti dsDNA IgG antibodies present in the original sample.

The concentration of the anti dsDNA IgG antibodies in the sample are calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

- Calibrators** (5 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1 M, NaN₃ < 0.1%, human serum
CAL0 **REF DCE002/9506-0**
CAL1 **REF DCE002/9507-0**
CAL2 **REF DCE002/9508-0**
CAL3 **REF DCE002/9509-0**
CAL4 **REF DCE002/9510-0**
- Controls** (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1 M, NaN₃ < 0.1%, human serum
Negative Control **REF DCE045/9501-0**
Positive Control **REF DCE045/9502-0**
- Sample Diluent** (1 vial, 100 mL)
Phosphate buffer 0.1 M, NaN₃ < 0.1%
REF DCE053/9553-0
- Conjugate** (1 vial, 15 mL)
Anti h-IgG conjugated with peroxidase, BSA 0.1%,
Proclin < 0.0015% **REF DCE002/9502-0**
- Coated Microplate** (1 breakable microplate)
Microplate coated with dsDNA **REF DCE002/9503-0**
- TMB Substrate** (1 vial, 15 mL)
H₂O₂ -TMB (0,26 g/L) (avoid any skin contact)
REF DCE004-0
- Stop Solution** (1 vial, 15 mL)
Sulphuric acid 0.15M (avoid any skin contact)
REF DCE005-0
- 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, Calibrators and 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 (NaN₃) 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/H₂O₂ 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 lipemic 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 (C₀...C₄)

Calibration curve is ready to use and is calibrated against International Standard WHO Wo/80. The Calibrators have approximately the following concentration:

	C ₀	C ₁	C ₂	C ₃	C ₄
IU/mL	0	12.5	25	50	200

NB: Calibrators concentration is lot-dependent; exact concentrations are indicated on Certificate of Analysis and labels.

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

6.2. Preparation of the Sample

For determination of anti dsDNA 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.

The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation.

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.

Neither Bilirubin nor Hemolysis have significant effect on the procedure.

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 (C₀-C₄), 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 45 minutes at 37°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 45 minutes at 37°C. Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution (if you use automated equipment, wash the wells at least 5 times). 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 test Anti dsDNA IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. 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

Following ranges have been established with the Anti dsDNA IgG tests:

	Anti dsDNA IgG (IU/mL)
Normal	< 25
Elevated	> 25

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 Manufacturer 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 dsDNA.

9. PERFORMANCE AND CHARACTERISTICS

9.1. Specificity

Comparison test against a commercial reference kit, performed on 100 sera (50 of them positive sera and 50 negative sera) showed a specificity > 99%.

9.2. Sensitivity

Comparison test against a commercial reference kit, performed on 100 sera (50 of them positive sera and 50 negative sera) showed a sensibility > 99%..

9.3. Detection limit

The lowest concentration of Anti dsDNA IgG that can be distinguished from zero Calibrator is 0.135 IU/mL.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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