Anti dsDNA IgG

GWB-521215

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FOR RESEARCH USE ONLY

Enzyme immunoassay for the quantitative determination of anti dsDNA in human serum or plasma

1. 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.

2. PRINCIPLE OF THE ASSAY

Anti-dsDNA IgG test is based on the binding of serum or plasma IgG antibodies on dsDNA coated on the microplates. The antibodies in calibrators, controls or prediluted samples bind into the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive serum components. An anti-human-IgG horseradish peroxidase conjugate solution recognizes the IgG class antibodies bound to the immobilized dsDNA antigens. After a 30 minutes incubation any excess of enzyme conjugate, which is not specifically bound, is washed away with wash buffer. 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.

3. MATERIALS

3.1. Reagents supplied

Anti dsDNA Coated Wells: 12 breakapart 8-well snap-off strips coated with dsDNA; in resealable aluminum foil.
Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.25 mol/l (avoid any skin contact), ready to use
Conjugate: 1 bottle containing 15 ml with anti h-IgG conjugated with horseradish peroxidase (HRP)
TMB Substrate Solution: 1 bottle containing 15 ml 3, 3´, 5, 5´-tetramethylbenzidine (H_2O_2-TMB 0.26 g/l) (avoid any skin contact), ready to use
Sample diluent: 1 bottle containing 100ml, Phosphate buffer
Wash solution: 1 bottle containing 50 ml (10x conc.)
anti-dsDNA Standards: 5 bottles, 1.2 ml each, ready to use
Standard 0: 0 AU/ml
Standard 1: 15 AU/ml
Standard 2: 30 AU/ml
Standard 3: 60 AU/ml
Standard 4: 240 AU/ml
Negative Control: 1 bottle containing 1.2 ml, ready to use
Positive Control: 1 bottle containing 1.2 ml, ready to use

3.2. Materials supplied

1 Strip holder
1 Cover foils
1 Test protocol
1 Distribution and identification plan

3.3. Materials and Equipment needed

ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
Manual or automatic equipment for rinsing wells
Pipettes to deliver volumes between 10 and 1000 µl
Vortex tube mixer
Distilled water
Disposable tubes
Timer

4. STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.

5. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

5.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with dsDNA antibodies. Store at 2…8 °C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.*

5.2. anti-dsDNA Standards/controls

The assay system is calibrated in relative arbitrary units. The standards have approximately the following concentration:

	S0	S1	S2	S3	S4
AU/mL	0	15	30	60	240

5.3. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away*.

5.4. Stop Solution

The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

5.5. 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. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

6. SPECIMEN COLLECTION AND PREPARATION

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. Therefore 10 L of sample may be diluted with 990L of sample diluent . 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.

7. ASSAY PROCEDURE

7.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well (e.g. A1) for the substrate blank 2 wells (e.g. B1+C1) control negative 2 wells (e.g. D1+E1) for standard 0 2 wells (e.g. F1+G1) for standard 1 2 wells (e.g. H1+A2) for standard 2 2 wells (e.g. B2+C2) for standard 3 2 wells (e.g. D2+E2) for standard 4 2 wells (e.g. F2+G2) control positive

7.2. Measurement

Reagent	Standard	Sample or Controls	Blank
Standard S0-S4	**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 three times with 300 µL of diluted wash solution			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells three times with 300 µL of diluted wash solution.			
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 Blank.			

8. RESULTS

8.1. Calculation of results

For 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.

Typical Results (example only)The figure below show typical results for Anti-dsDNA IgG. These data are intended for illustration only and should not be used to calculate results from another run.

N	OD1	OD2	mean	C1	C2	mean	CV%
STD0	0.007	0.008	0.008	0.00	0.03	0.01	
STD1	0.426	0.403	0.415	15.90	14.97	15.44	4.28
STD2	0.731	0.717	0.724	29.68	28.98	29.33	1.68
STD3	1.208	1.243	1.226	59.17	61.90	60.53	3.19
STD4	2.272	2.218	2.245	251.3	229.3	240.3	6.48

9. SPECIFIC PERFORMANCE CHARACTERISTICS

9.1.Precision and reproducibility

Intra-Assay
Within run variation was determined by replicate 16 times two different sera with values in the range of standard curve. The within assay variability is ≤3.4%
Inter-Assay
Between run variation was determined by replicate measurements of two different control sera with different lots of kits and/or different mix of lots

of reagents. The between assay variability is ≤11.7%.

9.2.Specificity / Sensitivity

Comparison test against a commercial reference kit, performed on 36 sera (15 of them positive sera and 21 negative sera) showed a 95.24% specificity.

Comparison test against a commercial reference kit, performed on 36 sera (15 of them positive sera and 21 negative sera) showed a 93.33% sensibility.

9.3.Detection Limit:

The lowest concentration of anti-dsDNA IgG that can be distinguished from zero standard is 0.42 AU/mL with a confidence limit of 95%.

10. PRECAUTIONS AND WARNINGS

WARNINGS

This kit is intended for research use only by professional persons.
Use appropriate personal protective equipment while working with the reagents provided.
All human source material used in the preparation of standards and controls for this product 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 Standard 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 (NaN3) or Proclin 300R 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.

PRECAUTIONS

Please adhere strictly to the sequence of pipetting steps provided in this protocol.
All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly 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 dates printed on the labels of the box and of the vials 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.
If you use automated equipment is your 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.
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 laboratories by assaying controls and/or pooled sera.
Maximum precision is required for reconstitution and dispensation of the reagents.
Samples microbiologically contaminated should not be used in the assay. Highly lipemeic or haemolysed specimens should similarly not be used
Plate readers measure vertically. Do not touch the bottom of the wells.

11. LITERATURE

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SCHEME OF THE ASSAY

Anti-dsDNA

Test Preparation

Prepare reagents and samples as described.Establish the distribution and identification plan for all specimens and controls on the resultsheet supplied in the kit.

Select the required number of microtiter strips or wells and insert them into the holder.

Reagent	**Standard**	**Sample or Controls**	**Blank**
Standard S_0-S_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 three times with 300 µL of diluted wash solution			
Conjugate	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells three times with 300 µL of diluted wash solution.			
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 Blank.			

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