ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Anti-dsDNA ELISA (IgG)

(Catalog Number: 31A040)

For the quantitative determination of human anti-dsDNA ELISA (IgG class antibodies) in serum or plasma

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

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INTRODUCTION

Antibodies against DNA are distinguished into two different types: antibodies against double stranded, native DNA (dsDNA) and antibodies against single-stranded, denatured DNA. The dsDNA-specific antibodies (anti-dsDNA) are considered a specific marker for systemic lupus erythematosus (SLE), due to the high clinical associations[1-3]. The presence of these autoantibodies could be virtually diagnostic for SLE[1,3]. In fact, anti-dsDNA antibodies may be present in patients even before they develop clinical features of SLE[2,4]. Therefore, monitoring the condition of anti-dsDNA antibodies is essential for maintaining the healthy condition as well as identifying the progression of SLE. Moreover, the identification of anti-dsDNA antibodies in other pathological conditions and in healthy subjects is very rare (less than 0.5%)[3].

PRINCIPLE OF THE ASSAY

The microtiter plate is pre-coated with double-stranded DNA (dsDNA) antigens. Standards and samples are added into the wells and any dsDNA-specific antibodies present in the sample is bound by the immobilized antigens. After washing, any unbound antibodies will be removed. Then, goat anti-human IgG-horseradish peroxidase (HRP) conjugate is added. The conjugate binds to the captured dsDNA-specific antibodies. Then substrate is catalyzed by the HRP to produce a blue color that changes to yellow after adding the stopping buffer. The density of the yellow coloration is directly proportional to the amount of captured dsDNA-specific antibodies in the plate. The light absorbance (OD value) under 450nm wavelength of the wells is determined using a microplate reader. The antibody concentration of the unknown sample can be estimated with the provided calibrators in the kit, which are calibrated against International Standard WHO Wo/80. The kit offers **semiquantitative** and **quantitative** interpretation of the data, which is in the section of **DATA INTERPRETATION**.

INTENDED USE

Human Anti-dsDNA ELISA (IgG) is an *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit for the accurate quantitative measurement of IgG class antibodies against dsDNA in human serum or plasma.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. ELISA plate, covered with dsDNA antigens for detecting human sourced dsDNA autoantibodies, 12 strips (8 wells/strip), sealed
2. 5×Sample buffer, 12 mL
3. Calibrator 1 (30 IU/mL)
4. Calibrator 2 (200 IU/mL)
5. Calibrator 3 (600 IU/mL)
6. Positive control, human sourced, ready for use
7. Negative control, human sourced, ready for use
8. 10×Wash buffer, 50 mL
9. Goat anti-human IgG-HRP solution, 12 µL, ready for use

10. Substrate solution, 12 mL, ready for use
11. Stopping solution, 2 M H_2SO_4, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes or pipette tips
2. Microplate washer
3. Buffer and reagent containers
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbance at 450 nm
6. Distilled/deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips can be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. Sample preparation

Vortex and centrifuge the sample tubes with a microcentrifuge at 16,000×g for 1 minute. Dilute the sample (serum or plasma) into sample buffer following the ratio of 1:101, and store under 2-8°C before further usage.

B. Calibrators, Positive control and Negative control

The calibrators and controls are diluted with sample buffer following the ratio of 1:101, and store under 2-8°C before further usage. Calibrators used for generating the standard curve are calibrated against International Standard WHO Wo/80.

C. 1×Wash buffer

Prepare 1×Wash buffer by diluting the 10×Wash buffer (50 mL) with 450 mL of distilled/deionized water (v/v = 1:9). If crystals are observed in the 10×Washing buffer bottle, incubate the bottle in a 37°C water bath until the crystals is fully dissolved and further vortex the bottle for 1 minute. The 1×Wash buffer can be stored at 2-8°C for up to one month.

D. Goat anti-human IgG-HRP solution

Vortex the bottle to ensure the liquid is fully mixed before use. Dilute the anti-human IgG-HRP in 1×Sample buffer. Once the bottle is open, store at 2-8°C.

E. Substrate solution

Substrate solution is ready for use. As the solution is highly sensitive to the light, ensure the bottle is fully closed after use. The solution is clear and colorless. Dispose the solution if it turns blue.

F. Stopping solution

Stopping solution contains 2 M H_2SO_4, ready for use.

ASSAY PROCEDURE

It is recommended that all standards and samples be run with blank wells and in duplicate.

1. Add 100 µL of calibrator, positive control, negative control or sample dilution into each well, incubate at room temperature (around 23°C) for 30 minutes.
2. Discard the content and tap the plate on a clean paper towel to remove residual liquid in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
   Note: When the residual in the well (>10 µL) can interfere the reaction between the reagents, leading to a lower OD value. The inadequately washing (e.g., less than 3 repeats, inadequate wash buffer or washing for a short period of time) of the plates can cause a higher OD value.
3. Add 100 µL of diluted Goat anti-human IgG-HRP solution to each well, incubate at room temperature (around 23°C) for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µL of Substrate solution to each well (e.g., 5 seconds between two wells), incubate at room temperature for 5 minutes.
6. Add 100 µL of Stopping solution to each well with the same pace as adding the substrate (e.g., 5 seconds between two wells), gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm within 30 minutes.

DATA INTERPRETATION

- **Semi-quantitative:**

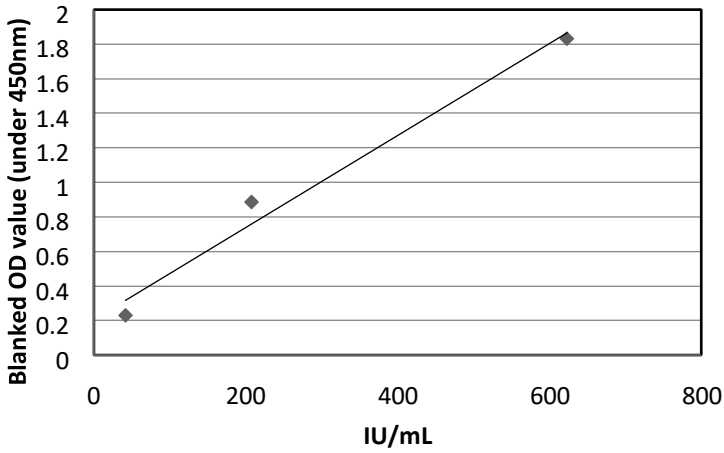
Comparing the OD value of the sample with the calibrators:

> Calibrator 1 – suggestion for self-monitoring
> Calibrator 2 – suggestion for seeking a doctor
> Calibrator 3 – suggestion for treatment

- **Quantitative:**

1. Subtract the absorbance of the blank wells from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (OD value, y-axis) against the concentration of the 3 Calibrators (IU/mL, x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine dsDNA antibody concentrations of samples from standard curve.
4. The cutoff value is set to 25 IU/mL.

TYPICAL STANDARD CURVE



ASSAY CHARACTERISTICS

A. Linearity

The linearity of Anti-dsDNA ELISA (IgG) was determined by assaying 8 serial dilutions of 5 serum samples. The linear regression was calculated, R_2 amounting to >0.97 within the concentration range of 5 IU/mL to 600 IU/mL.

B. Reproducibility

The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different plates.

	Intra-assay variation, n=20		Inter-assay variation, n=4 x 6	
Serum	**Mean value (IU/mL)**	**CV (%)**	**Mean value (IU/mL)**	**CV (%)**
1	375	2.5	394	2.6
2	120	8.4	144	8.4
3	73	9.3	81	9.4

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5. M. R. ARBUCKLE, M. T. MCCLAIN, M. V. RUBERTONE ET AL., "DEVELOPMENT OF AUTOANTIBODIES BEFORE THE CLINICAL ONSET OF SYSTEMIC LUPUS ERYTHEMATOSUS," THE NEW ENGLAND JOURNAL OF MEDICINE, VOL. 349, NO. 16, PP. 1526–1533, 2003.

SUMMARY OF ASSAY PROCEDURE

Dilute the sample with Sample buffer (1:100).
↓
Add 100 µL of calibrators, controls or samples to each well.
↓
Incubate at room temperature for 30 minutes.
↓
Discard all the liquid.
↓
Wash the wells with 300 µL of 1×Wash buffer for 3 repeats.
↓
Add 100 µL of Goat anti-human IgG-HRP solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Discard all the liquid.
↓
Wash the wells with 300 µL of 1×Wash buffer for 3 repeats.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 5 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
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Data interpretation