Version 21-04/24

User's Manual

dsDNA IgG ELISA Kit

REF **DEIA277**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The present enzyme-linked immuno sorbent assay (ELISA) is intended for the quantitative or qualitative determination of IgG antibodies directed against dsDNA in human serum or plasma. The immobilised antigen is a highly purified preparation of plasmid dsDNA (90% in supercoiled state) free from chromosomal DNA and protein (histones). The test is fast (incubation time 30/ 30/30 minutes) and flexible (divisible solid phase, ready-lo-use reagents). Six calibrators allow quantitative measurements; a negative and a positive control check the assay performance.

General Description

Systemic lupus erythematosus (SLE) is an autoimmune-mediated, chronic inflammatory illness with variable clinical presentation, ranging from localised skin lesions to a destructive systemic disorder without cutaneous changes (1). Antibodies to double-stranded (ds)DNA are a well-known, specific marker for SLE with a prevalence from 50-90%, depending on disease severity (2, 3, 4). Their titre often correlates with disease activity (5). Circulating Anti-DNA/-DNA immune complexes are considered to play a role in the pathogenesis of SLE (6). dsDNA-antibodies constitute a SLE criterion according to the American Rheumatism Association (ARA).

Principles of Testing

The wells of the solid phase are coated with dsDNA. On this surface, the following immunological reactions take place:

1st reaction: dsDNA-specific antibodies present in the sample bind to the immobilised antigen, forming the antigen-antibody complex. Then, non-bound sample components are washed away from the solid phase.

2nd reaction: A second antibody, directed at human IgG antibodies and conjugated with horse-radish peroxidase (HRP), is added. This conjugate binds to the complex. Then, excess conjugate is washed away from the solid phase.

3rd reaction: The enzyme-labelled complex converts a colourless substrate into a blue product. The degree of colour development reflects the concentration of dsDNA IgG in the sample.

Reagents And Materials Provided

1. **MTP** 1 Microtiter Plate, coated with dsDNA and hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.
2. **ENZCONJ IgG** Enzyme Cconjugate IgG, 14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.
3. **CAL A-F** 6 Calibrators, 2.0 mL each, 0 - 6.5 - 16 - 40 - 100 and 250 IU dsDNA IgG / mL, ready-to-use, gradually blue coloured. Contain TBS, BSA, Tween and Na-azide.
4. **CONTROL -and CONTROL +** Negative and Positive Control, 2.0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.

5. **SAMPLEDIL** Sample Diluent, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.
6. **TMB SUBS** TMB Substrate Solution, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and H_2O_2. Contained in a vial impermeable to light.
7. **WASHBUF CONC** Wash Buffer, 100 mL, 10×-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.
8. **STOP** TMB Stop solution (0.2 M H_2SO_4), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.
9. Directions for use
10. Lot-specific certificate of analysis

Materials Required But Not Supplied

1. Deionised or distilled water
2. Graduated cylinder, 1000 mL
3. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)
4. Pipettes for 10, 100 and 1000 µL (1- and 8-channel pipettes recommended)
5. Microwell plate washer (optional)
6. Microwell plate photometer fitted with a 450 nm filter
7. ELISA evaluation program (recommended)

Storage

Store kit at 2-8°C. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Specimen Collection And Preparation

1. **Preparation of the samples:** handle patient specimens as potentially infectious agents. Besides serum, EDTA- or citrate-treated plasma are suitable sample material as well; heparin-treated plasma however is not.
2. **Specimen requirements:** highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.
3. Prepare samples using normal laboratory techniques. Turbid samples must first be clarified (centrifuged). The clarified or clear samples are mixed and then diluted 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Also mix the dilution.
4. For rapid dispensing during the assay procedure, preparation of the calibrators, controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure.
5. If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. For longer storage, -20°C or lower temperatures are recommended. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

Reagent Preparation

1. Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amount of reagents should be withdrawn. It is crucially important that no cross-contamination between the reagents occurs. Use only clean pipettes and do not pour back residues into the original flasks.
2. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.
3. Dilute the wash buffer 10×-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).

Assay Procedure

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C). To achieve best results, i.e. the maximum ratio between specific and background signal, **careful washing** is essential (steps 1, 3 and 5). It is **crucially important to remove the wash solution completely.** For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

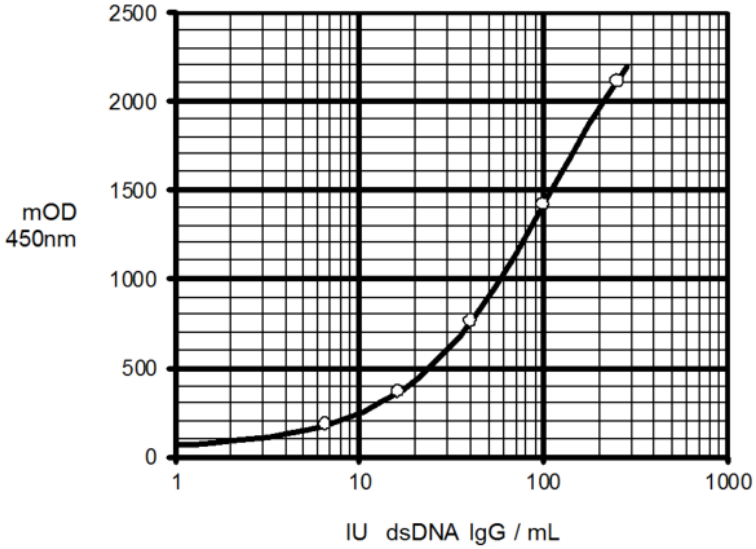
1. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.
2. Dispense the calibrators (2.0 mL each, ready-to-use, gradually blue), controls (2.0 mL each, ready-touse, green and red) and the diluted samples rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended. Incubate the plate for 30 minutes at room temperature (23 ± 3°C).
3. Wash the wells 4 times as in step 1.
4. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, red); 100 µL per well. Incubate the plate as in step 2.
5. Repeat wash step 3.
6. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use, colourless, black vial); 100 µL per well. Incubate the plate as in step 2. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.
7. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.
8. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

Evaluation

Quantitative evaluation: the data obtained are quantitatively evaluated with the standard curve, as shown below. However, the depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples. The curve has been constructed with a conventional ELISA evaluation program, using a 4-parameter function. The Spline approximation is also

appropriate.



If no computer-supported evaluation is possible, the standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into IU dsDNA IgG per mL sample.

Qualitative evaluation: the test may also be evaluated in a qualitative manner. This requires measurement of the positive control only. Nevertheless, measurement and examination of the negative control is recommended (see below: Quality control).

In qualitative test evaluation, the absorbance of the samples is compared with the borderline absorbance

(= cut-off). It is determined according to the following formula:

absorbance borderline = absorbance positive control × factor

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis which is included with each test kit. Example:

absorbance positive control= 1250 mOD

factor= 0.35

absorbance borderline = 1250 mOD × 0.35 = 438 mOD

In order to gain an impression of how positive a particular sample is for dsDNA IgG, one may calculate the ratio, according to the formula:

ratio = absorbance sample / absorbance borderline

Example:

Absorbanceborderline = 438 mOD

absorbancesample = 1480 mOD

ratio = 1480 mOD / 438 mOD = 3.4

Quality control: the positive and negative control check the assay performance. Their authorised values and acceptable ranges, respectively, are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

Performance Characteristics

1. Standardisation

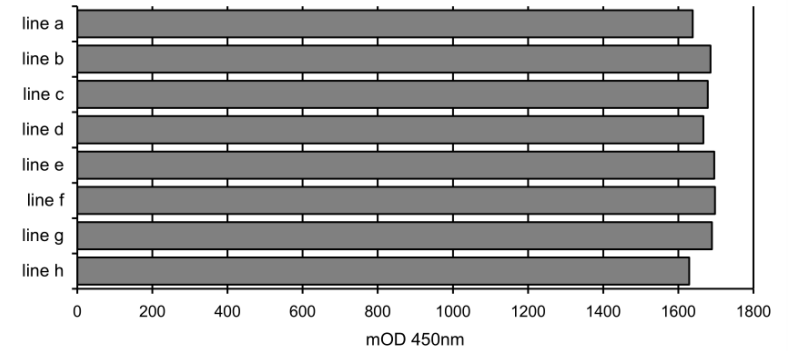
The test is standardised with a purified mAb preparation containing IgG antibodies specifically directed at dsDNA. This preparation has been calibrated against the first international standard for dsDNA-Ab, coded Wo/80. The degree of sample reactivity is measured in international units (IU dsDNA IgG / mL).

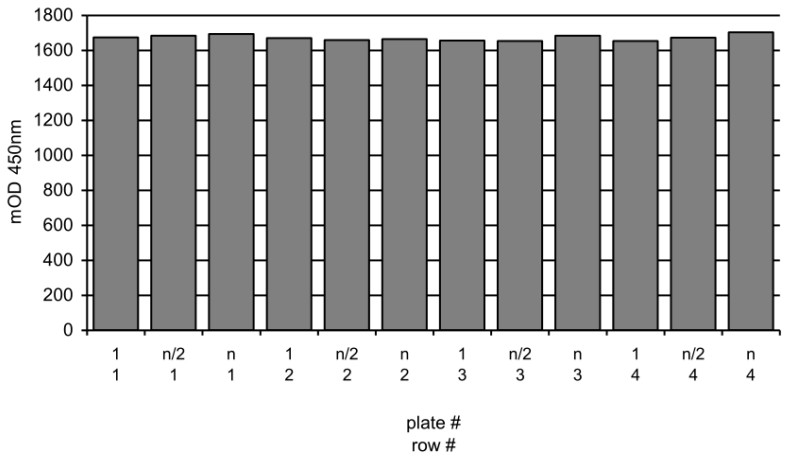
2.Homogeneity of the solid phase

Measurement of the solid phase homogeneity is a regular QC part of each production lot. This is determined by 288-fold measurement of a positive but non-saturating sample on 3 selected plates. Acceptance criterion: mOD-coefficient of variation (cv) over the plates < 8%.

The figure below shows a representative excerpt (solid phase lot no. 2210R) of such an analysis.

plate row	1 1	n/2 1	n 1	1 2	n/2 2	n 2	1 3	n/2 3	n 3	1 4	n/2 4	n 4	mean	cv %
line a	1663	1644	1678	1643	1607	1644	1676	1629	1658	1608	1592	1615	1638	1,7
line b	1684	1682	1674	1640	1671	1696	1683	1687	1712	1687	1707	1710	1686	1,2
line c	1672	1710	1677	1678	1654	1669	1684	1660	1732	1643	1668	1697	1679	1,5
line d	1633	1668	1671	1668	1668	1657	1648	1649	1679	1653	1681	1729	1667	1,4
line e	1703	1696	1720	1694	1691	1689	1658	1661	1700	1681	1728	1727	1696	1,3
line f	1693	1726	1724	1699	1683	1685	1661	1676	1705	1696	1693	1732	1698	1,3
line g	1720	1700	1713	1689	1684	1668	1641	1678	1677	1675	1707	1722	1690	1,4
line h	1631	1647	1689	1656	1615	1608	1597	1589	1614	1587	1613	1700	1629	2,3
mean	1675	1684	1693	1671	1659	1665	1656	1654	1685	1654	1674	1704	**1673**	
cv %	1,9	1,7	1,3	1,4	1,9	1,7	1,7	1,9	2,2	2,4	2,9	2,2		**2,1**





Precision

For the assessment of the test precision, the variability of results under the following conditions was determined: a. within 1 assay and between 3 assays, b. between 3 operators and c. between 2 kit lots.

a. Intra- and inter-assay variability (n = 24 and 72, respectively)

sample	mean IU/mL	variability (cv, %) intra-assay	inter-assay
1	23	3,3	3,5
2	82	2,2	3,1
3	100	2,0	2,0

b. Operator to operator variability (n = 12)

sample	mean IU/mL	variability (cv, %)
1	27	4,1
2	130	2,1
3	160	2,4

c. Variability between 2 kit lots (n = 6)

sample	mean IU/mL	variability (cv, %)
1	27	8,0
2	94	6,0
3	110	6,4

Detection Limit

The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of Sample Diluent plus 3-fold standard deviation (s). It was determined as < 1 IU dsDNA IgG per mL sample (n = 24).

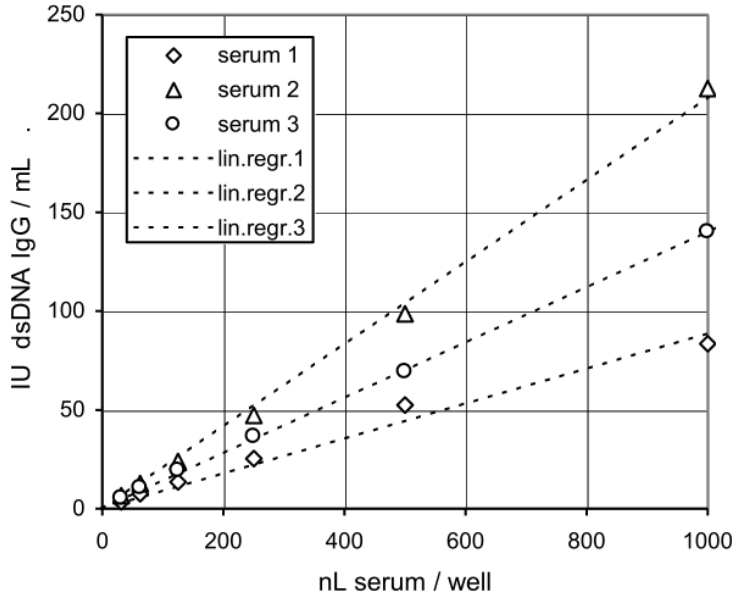
Recommended measuring range: 5 – 250 IU dsDNA IgG per mL sample

Specificity

The test allows the specific determination of human IgG antibodies directed against dsDNA.

Linearity

In order to assess the dose-response relationship of the test, positive sera were measured in serial 2-fold dilution. Acceptance criterion: linear regression of 4 successive dilutions must yield a correlation factor> 0.98. A typical result is depicted below.



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