Version 22-04/24

User's Manual

Anti-dsDNA (Mouse) IgM ELISA Kit

REF **DEIA-PY07130**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The ELISA Kit is designed for quantitative and rapid measurement of mouse anti-dsDNA IgM antibody levels in serum or plasma samples.

General Description

High levels of serum autoantibodies against deoxyribonucleic acid (DNA) are observed in most patients with systemic lupus erythematosus (SLE) (1, 2), therefore the presence of anti-DNA antibodies in serum is considered a valuable marker for the diagnosis of SLE. Moreover, the serum anti-DNA antibodies form anti-DNA/DNA immune complexes which play an important role in the immunopathogenesis of lupus nephritis caused by SLE (3). Anti-single stranded DNA (ssDNA) IgG antibodies are elicited in the early stages of SLE, whereas anti-double stranded DNA (dsDNA) IgG antibody levels correlate with the severity of SLE. On the other hand, anti-dsDNA lgM antibodies are not specific to SLE, but correlate with the prognosis of lupus nephritis in patients with SLE (4, 5). Therefore, evaluating immunoglobulin levels of different isotypes against individual DNA types may signify a particular stage and the prognosis of SLE.

Mouse SLE models, which translate relevant information to the human condition, elucidate the cellular and genetic requirements. For example, in spontaneous mouse NZB/W F1 lupus models, anti-dsDNA antibody isotype class switching from lgM to IgG indicates renal failure which is observed in human SLE (6). Nonetheless, in artificial pristane-induced Balb/c lupus models, anti-ssDNA lgM antibodies solely induce SLE (7-9). Therefore, to study the diverse roles of anti-DNA antibodies in these mouse SLE models, Creative Diagnostics, Inc. provides anti-dsDNA lgM (Catalog # DEIA-PY07130) antibody ELISA kits.

Reagents And Materials Provided

1. Standard Mouse anti-DNA IgM Antibody(1 vial): 50 ng/vial, 100 µL.
2. dsDNA(1 vial): 0.5 mg/mL, 100 µL.
3. Secondary Antibody, Peroxidase-Conjugated Goat Anti-Mouse IgM Polyclonal Antibody (2 vials): 50 µL.
4. Solution A - Coating Buffer (1 bottle): 10 mL.
5. Solution B - Blocking Buffer (1 bottle): 10 mL.
6. Solution C - Sample/Standard/Secondary Antibody Dilution Buffer (1 bottle): 50 mL.
7. TMB Solution (2 vials): 0.2 mL.
8. Chromogen Dilution Buffer (1 bottle): 20 mL.
9. Stop Solution - 2N Sulfuric Acid (1 bottle): 10 mL.
11. Wash Buffer, 20X (1 bottle): 50 mL.
12. ELISA Plate 1 each 96-well(8-well strips x 12)

Materials Required But Not Supplied

1. Precision pipettes and tips

2. Distilled or deionized water
3. Polypropylene or glass tubes
4. Vortex mixer
5. Absorbent paper or paper towels
6. Plate reader with an optical density range of 0-4 at 450nm

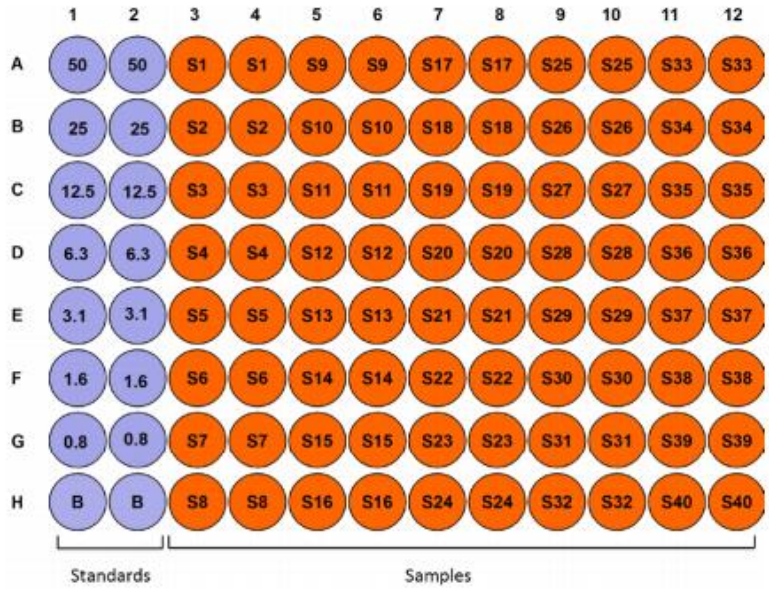
Storage

All the kit components should be stored at -20°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize

exposure to damp air.

Plate Preparation

Figure 1. A Standard Assay Layout



Reagent Preparation

Wash Buffer:

Dilute 50 mL of 20X wash buffer in 950 mL of distilled water (1X wash buffer).

Secondary Antibody Solution:

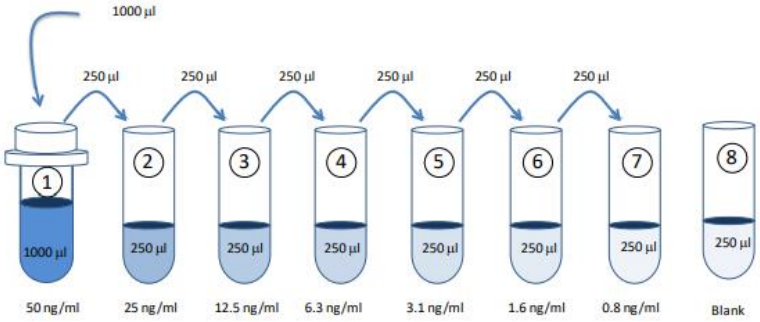
Centrifuge the Secondary Antibody vial at 3000 rpm x 1 minute. Dilute one vial of Secondary Antibody in 10 mL of Sample/Standard/Secondary Antibody Dilution Buffer (Solution C).

TMB Solution:

Use new tubes when preparing TMB. Centrifuge the TMB Solution vial at 3000 rpm x 1 minute. Dilute one vial of TMB with 10 mL of Chromogen Dilution Buffer just prior to use.

Prepare Standard Dilutions:

The recommended standard range is 0.8-50 ng/mL. Dissolve one vial of Standard (50 ng/vial) in 1 mL of Sample/Standard/Detection Antibody Dilution Buffer (Solution C) and keep it as a standard stock. Then, serially dilute it with Solution C. For example, mix 250 µL of the 50 ng/mL solution with an equal volume of Solution C to make a 25 ng/mL solution, and then repeat it five more times for 12.5, 6.3, 3.1, 1.6, and 0.8 ng/mL standard solutions.

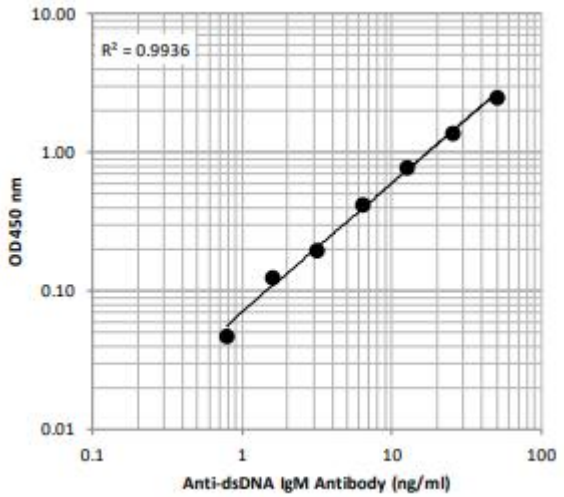


Assay Procedure

1. Add DNA Solution: Centrifuge the DNA Solution tube at 3000 rpm x 1 minute. Dilute one vial of DNA with 10 mL of Solution A. Add 100 µL of DNA solution to each well and incubate at 4°C overnight.
2. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.
3. Add Blocking Buffer: Add 100 µL of the Blocking Buffer (Solution B) to each well and incubate at room temperature for 1 hour.
4. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.
5. Add Standards and Samples: Add 100 µL of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.
6. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.
7. Add Secondary Antibody: Add 100 µL of secondary antibody solution to each well and incubate at room temperature for 1 hour.
8. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.
9. Add TMB: Add 100 µL of TMB solution to all wells immediately after washing the plate and incubate for 25 minutes at room temperature.
10. Stop: Add 50 µL of 2N sulfuric acid (Stop Solution) to each well.
11. Read Plate: Read the OD values at 450 nm (a 630 nm filter can be used as a reference). If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

Calculation

1. Average the duplicate OD values for the standards, blanks (B), and test samples.
2. Subtract the averaged blank (B) values from the averaged OD values of the standards and test samples.
3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data.
4. The ng/mL of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration (ng/ml) in original sample specimens.



Precision

Standard was added with known amounts of anti-dsDNA IgM antibodies and then diluted with Sample/Standard/Secondary Antibody Dilution Buffer to assay anti-dsDNA IgM antibodies by ELISA.

Test At	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Inter-Assay CV (%)	8.6	7.6	6.0
Intra-Assay CV (%)	8.1	4.3	0.1
Spiking Test*	98.3 %	92.9 %	94.4 %

Detection Range

0.8 ng/mL to 50 ng/mL

Precautions

1. It is recommended that the standard and samples be run in duplicate.
2. Warm up all buffers to room temperature before use.
3. Partially used reagents may be kept at –20°C.
4. Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.
5. Measure exact volume of buffers using a serological pipette, as extra buffer is provided.

6. Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.
7. This kit contains components of animal origin from non-infectious animals, but should be treated as potential biohazards in use and for disposal.

References

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