User's Manual

Anti-dsDNA (Mouse) IgM ELISA Kit

REF **DEIA4488M**

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.

PRODUCT INFORMATION

Intended Use

For detection of Anti-dsDNA (Mouse) IgM in test samples.

General Description

High levels of serum autoantibodies against deoxyribonucleic acid (DNA) are observed in most patients with systemic lupus erythematosus (SLE), therefore the presence of anti-DNA antibodies in serum is considered a valuable marker for the diagnosis of SLE. Especially considering that the serum anti-DNA antibodies form anti-DNA/DNA immune complexes which play important roles in the immunopathogenesis of renal injury also known as lupus nephritis. With regards to the specificity of anti-DNA antibodies, 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 IgM antibodies are not specific to SLE, but correlate with the prognosis of lupus nephritis in patients with SLE. Therefore, evaluating immunoglobulin levels of different isotypes against individual DNA types may indicate the stages and prognosis of SLE. Mouse models, which provide relevant information to the human condition, elucidate the cellular and genetic requirements for inducing SLE. For example, in spontaneous murine NZB/W F1 lupus models, anti-dsDNA antibody isotype class switching from IgM to IgG indicates renal failure which is a similar trend in human SLE. Nonetheless, in artificial pristane-induced Balb/c lupus models, anti-ssDNA IgM antibodies solely induce SLE. In order to advance studies in these fields, CD provides mouse anti-DNA antibody ELISA kits to elucidate the diverse roles of anti-DNA antibodies in these mouse SLE models.

Reagents And Materials Provided

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

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm

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Tel: 1-631-624-4882 (USA)   **Fax:** 1-631-938-8221

Tel: 44-161-818-6441 (Europe)   **Email:** info@creative-diagnostics.com

2.   Data reduction software

3.   Multi-channel dispenser or repeatable pipette for 100 µl

4.   Vortex mixer

5.   Pipettes for 10 µl, 100 µl and 1000 µl

6.   Laboratory timing device

7.   Distilled or deionised water

8.   Measuring cylinder for 1000 ml and 100 ml

9.   Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system.

Storage

-20°C for 12 months

Plate Preparation
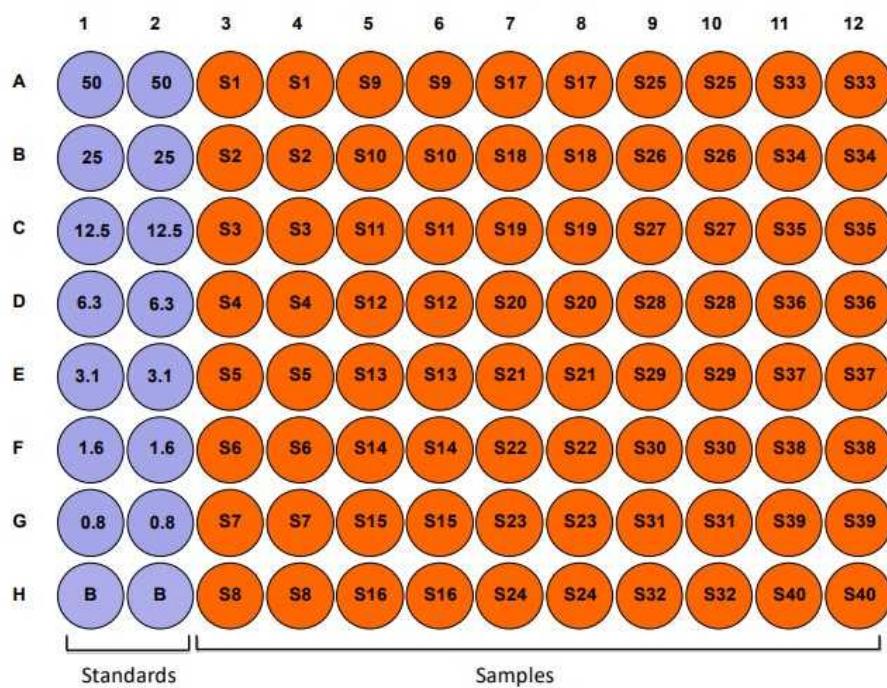
Assay Procedure

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20°C for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

1. Add DNA Solution: 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.

Strip #	DNA (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. Dilute Wash Buffer: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). 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 blotting 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. 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/Secondary Antibody Dilution Buffer (Solution C) and keep it as a standard stock solution. 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.
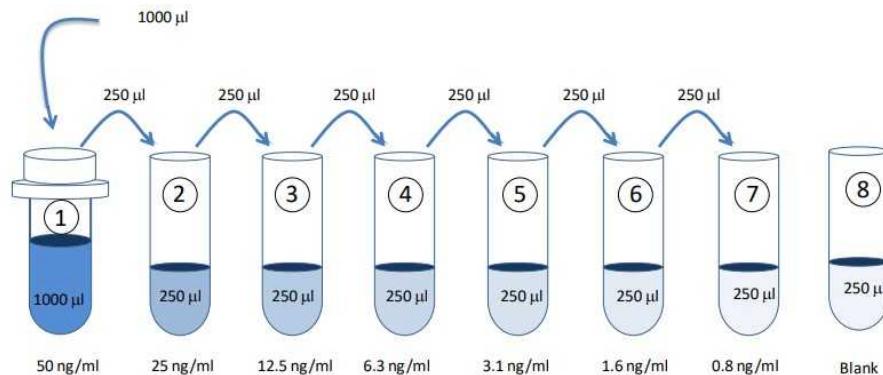
5. Prepare Sample Dilutions: Mouse serum dilution varies (1:100 or more) depending on the animal model and timing of serum collection. In general, no IgM antibodies against DNA are observed in normal serum at a 1:100 dilution.

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 blotting on a paper towel to remove excess liquid. Do not allow the plate to dry out.

7. 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.

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 blotting on a paper towel to remove excess liquid. Do not allow the plate to dry out.

9. Add Secondary Antibody: Dilute one vial of Secondary Antibody in 10 ml Sample/Standard/Secondary Antibody Dilution Buffer (Solution C). Add 100 μl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	2nd Antibody (μl)	Solution C (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

10. 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 blotting on a paper towel to remove excess liquid. Do not allow the plate to dry out.

Strip #	TMB (μl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

12. Stop: Stop the reaction with 50 μl of 2N Sulfuric Acid (Stop Solution) to each well.

13. Read Plate: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, reassay the samples at a higher dilution. A 630 nm filter can be used as a reference.

11. Add TMB Solution: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 μl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

Calculation

1. Average the duplicate OD values for the standards, blanks (B), and test samples.
2. Subtract the averaged blank OD 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. Figures 1a and 1b show examples of standard curves for anti-DNA IgM antibodies.
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 the original test samples.

Figure 1a - A Typical Standard Curve for the anti-dsDNA IgM Antibody ELISA Kit
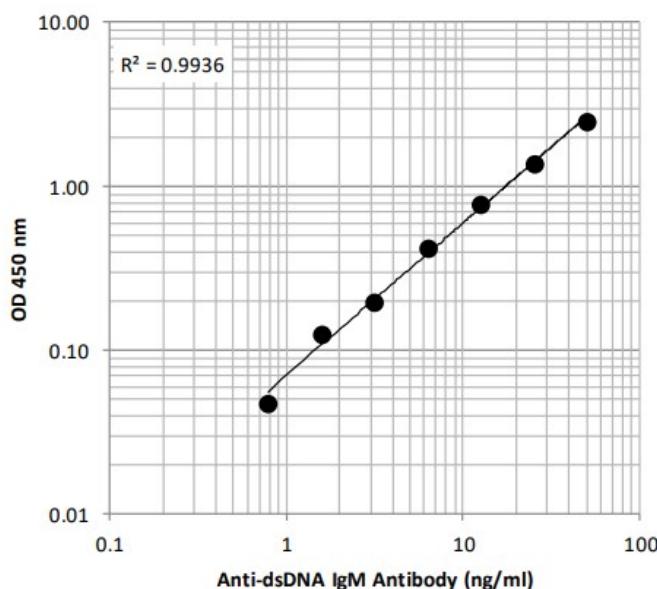

Figure 1b - A Typical Standard Curve for the Anti-ssDNA IgM Antibody ELISA Kit
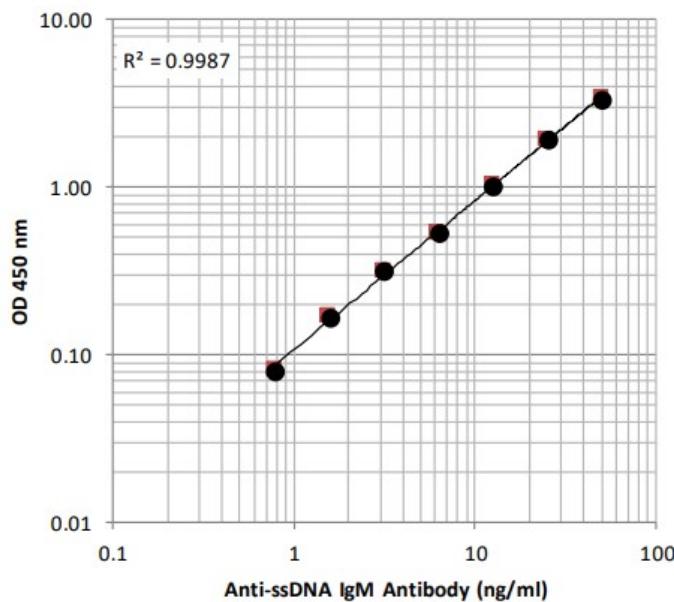
Detection Range

50 ng/ml to 0.8 ng/ml

Specificity

NA

Reproducibility

Intra-Assay (0.1-8.1%)/Inter-Assay (6.0-8.6%)/Spiking Test (92.9-98.3%)

Intra-Assay (2.7-6.3%)/Inter-Assay (2.7-7.0%)/Spiking Test (96.4-101.3%)

Table 1 - Reproducibility Data for the Mouse Anti-dsDNA IgM Antibody ELISA Kit

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

Table 2 - Reproducibility Data for the Mouse Anti-ssDNA IgM Antibody ELISA Kit

Test	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Intra-Assay CV (%)	6.3	3.4	2.7
Inter-Assay CV (%)	6.8	7.0	2.7
Spiking Test*	99.2 %	96.4 %	101.3 %

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