Chondrex, Inc.

Mouse Anti-dsDNA IgG Antibody Assay Kit (Catalog # 3031)
Mouse Anti-ssDNA IgGAntibody Assay Kit (Catalog # 3041)

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

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. Especially as 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 (3). 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 (4, 5). 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 (6). Nonetheless, in artificial pristane-induced Balb/c lupus models, anti-ssDNA IgM antibodies solely induce SLE (7-9). Therefore, to study the diverse roles of anti-DNA antibodies in these mouse SLE models, Chondrex, Inc. provides anti-dsDNA IgG (Catalog# 3031), anti-ssDNA IgG (Catalog# 3041) antibody assay kits in addition to anti-dsDNA IgM (Catalog# 3032) and anti-ssDNA IgGM (Catalog# 3042).

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Mouse anti-DNA IgG monoclonal Antibody (30311)	1 vial	50 ng/vial, lyophilized	-20°C
ssDNA (30412) or dsDNA (30312)	1 vial	0.5 mg/ml, 100 µl	-20°C
Detection Antibody (Peroxidase-Conjugated Goat Anti-Mouse IgG Polyclonal Antibody) (30113)	2 vials	50 µl	-20°C
Solution A - Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution B - Blocking Buffer (30313)	1 bottle	10 ml	-20°C
Solution C - Sample/Standard/Detection Antibody Dilution Buffer (30314)	1 bottle	50 ml	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

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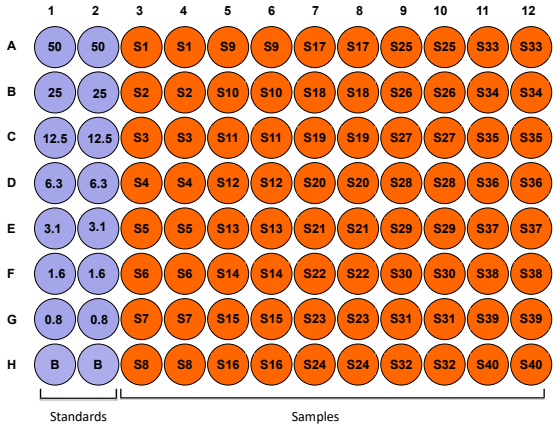
Figure 1. A Standard Assay Layout



NOTES BEFORE USING ASSAY

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: Partially used reagents may be kept at –20°C.

Note 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.

Note 5: Measure exact volume of buffers using a serological pipette, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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

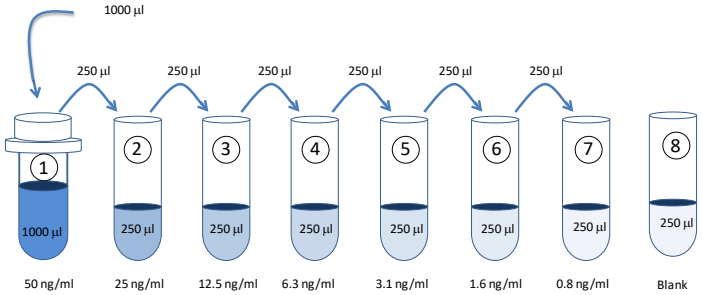
ASSAY PROCEDURE

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.

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 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. **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.



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

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

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

11. **Add TMB**: Use new tubes when preparing TMB. Dilute one vial of TMB with 10 ml Chromogen Dilution Buffer just prior to use. Add 100 µl of TMB solution to all wells immediately after washing the plate and incubate for 25 minutes at room temperature.

12. **Stop**: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

13. **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 OF ANTIBODY TITERS

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. Figures 2 and 3 show examples of standard curves for anti-DNA IgG 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 original sample specimens.

Figure 2 - A typical standard curve for anti-dsDNA IgG assay

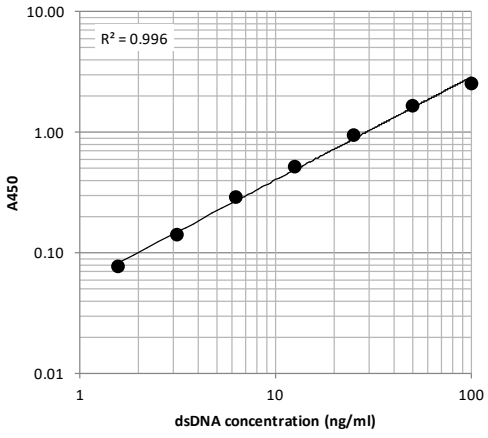

Figure 3 - A typical standard curve for anti-ssDNA IgG assay

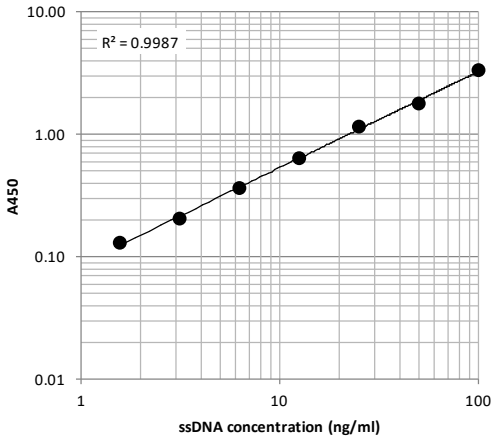

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Table 1 - Reproducibility of data assayed by Mouse Anti-dsDNA IgG Antibody Assay Kit

Test At	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Inter-Assay CV (%)	9.4	8.6	6.3
Intra-Assay CV (%)	9.9	9.5	3.9
Spiking Test*	104.0 %	106.0 %	93.4 %

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

Table 2 - Reproducibility of data assayed by Mouse Anti-ssDNA IgG Antibody Assay Kit

Test At	1.6 ng/ml	6.3 ng/ml	25 ng/ml
Inter-Assay CV (%)	9.8	9.5	7.5
Intra-Assay CV (%)	3.8	4.2	8.1
Spiking Test*	101.4 %	97.6 %	94.0 %

Standard was added to known amounts of anti-ssDNA antibodies and then diluted with Sample/Standard/Detection Antibody Dilution Buffer to assay anti-ssDNA IgG antibodies by ELISA.

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