Rat Anti-double-stranded DNA antibody; Natural DNA antibody (dsDNA-Ab) ELISA Kit

Cat #: orb3012514 (manual)

Size: 96 tests / 48 tests

For research use only, not for clinical diagnosis.

Introduction

Anti-double-stranded DNA antibodies (dsDNA-Ab) are a class of antinuclear antibodies (ANA) that target double-stranded DNA. They have strong diagnostic value for systemic lupus erythematosus (SLE) and are associated with the pathogenesis of lupus nephritis. dsDNA-Ab are produced when the immune system mistakenly recognizes self-DNA as foreign, leading to antibody binding, immune complex formation, and subsequent inflammation and tissue damage. As specific serological markers of SLE, dsDNA-Ab levels are closely associated with disease activity and have important diagnostic value, particularly in severe complications such as lupus nephritis. Dynamic monitoring of dsDNA-Ab levels can help evaluate treatment response, predict the risk of disease relapse, and guide personalized therapy.

Detection Principle

This kit is based on an indirect enzyme-linked immunosorbent assay (ELISA). Samples, positive controls, negative controls, and HRP-conjugated secondary antibodies are sequentially added to microwells pre-coated with double-stranded DNA (dsDNA)/native DNA antigen. After incubation and washing steps, TMB substrate is added for color development. TMB is converted to a blue-colored product under the catalytic action of horseradish peroxidase (HRP) and subsequently turns yellow after the addition of acid stop solution. The color intensity is positively correlated with the level of rat anti-double-stranded DNA/native DNA antibodies (dsDNA-Ab) present in the sample. The absorbance (OD value) is measured at 450 nm using a microplate reader to determine whether the sample is positive or negative.

Specificity: This assay specifically detects rat dsDNA-Ab with no significant cross-reactivity to similar substances.

Product Composition

Reagents	Specifications（96T）	Specifications（48T）
Pre-coated Assay Plate	8×12	8×6
Positive control	2 tubes	1 tube
Negative control	2 tubes	1 tube
Universal Diluent	2×20mL	1×20mL
HRP-antibody (100×)	120μL	60μL

Wash Buffer (20×)	2×10mL	1×10mL
TMB Substrate	10mL	5mL
Stop Solution	6mL	3mL
Plate Sealer	4 pieces	4 pieces

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision pipette with disposable tips: 0.5-10uL, 5-50uL, 20-200uL, 200-1000uL
3. 37°C incubator
4. Deionized or distilled water

Precautions

1. Strictly follow the specified incubation times and temperatures to ensure accurate results. All reagents must be brought to room temperature (20–25°C) before use. Store reagents at 2–8°C immediately after use.
2. Improper washing may lead to inaccurate results. Ensure that residual liquid is thoroughly removed from wells before adding substrate. Do not allow wells to dry out for extended periods during the procedure.
3. Clean any residual liquid or fingerprints from the bottom of the plate, as they may affect OD readings.
4. The substrate solution should be colorless. Do not use substrate that has turned blue.
5. Avoid cross-contamination between reagents and samples to prevent erroneous results.
6. Avoid exposure to strong light during storage and incubation.
7. Do not allow any reaction reagents to come into contact with bleach or strong oxidizing vapors, as these will destroy reagent activity.
8. Do not use expired reagents. Components from different lot numbers or kit types must not be mixed.
9. Recombinant proteins from other sources may not be recognized by the antibodies in this kit due to incompatibility.
10. All samples should be handled as potentially infectious and processed according to appropriate biosafety procedures.

Sample Collection and Requirements

1. The assay detection range does not necessarily reflect the concentration range of the analyte in samples. It is recommended to estimate the expected concentration from literature and confirm it through preliminary experiments. If the analyte concentration is too high or too low, dilute or concentrate the samples accordingly.
2. If the sample type is not listed in this manual, a preliminary experiment is recommended to validate its suitability.

3. **Serum:** Allow whole blood collected in a serum separator tube to clot at room temperature for 2 hours or at 2–8°C overnight. Centrifuge at 1,000 × g for 20 minutes and collect the supernatant. Store at -20°C or -80°C if not used immediately. Avoid repeated freeze–thaw cycles.

4. **Plasma:** Collect using EDTA or heparin as anticoagulant. Centrifuge within 30 minutes of collection at 1,000 × g for 15 minutes at 2–8°C. Collect the supernatant for testing or store at -20°C or -80°C. Avoid repeated freeze–thaw cycles.

5. **Tissue homogenate:** Rinse tissue with pre-chilled PBS (0.01 M, pH 7.4) to remove residual blood. Weigh and finely mince the tissue. Add PBS at a typical ratio of 1:9 (w/v), for example, 1 g tissue with 9 mL PBS (adjust as needed and record). Protease inhibitors are recommended. Homogenize thoroughly on ice using a glass homogenizer or mechanical homogenizer. Further disrupt cells by sonication or repeated freeze–thaw cycles if necessary. Centrifuge at 5,000 × g for 5–10 minutes and collect the supernatant for analysis.

6. **Cell culture supernatant:** Centrifuge at 1,000 × g for 20 minutes and collect the supernatant. Store at -20°C or -80°C if needed. Avoid repeated freeze–thaw cycles.

7. **Cell lysate**: For adherent cells, wash gently with pre-chilled PBS, detach with trypsin, and collect cells by centrifugation at 1,000 × g for 5 minutes. Suspension cells can be collected directly by centrifugation. Wash cells three times with pre-chilled PBS. Resuspend 1×10^6 cells in 150–200 µL PBS (protease inhibitors recommended; reduce volume if target concentration is low). Lyse cells by repeated freeze–thaw cycles or sonication. Centrifuge at 1,500 × g for 10 minutes at 2–8°C and collect the supernatant for analysis.

8. **Other biological specimens:** Centrifuge at 1,000 × g for 20 minutes and collect the supernatant for testing.

9. **Appearance of the sample:** Samples should be clear and transparent. Remove any particulate matter by centrifugation.

10. **Sample preservation:** Samples can be stored at 4°C if tested within one week. For longer storage, aliquot and freeze at -20°C (within 1 month) or -80°C (within 6 months). Avoid repeated freeze–thaw cycles. Hemolyzed samples may affect results and are not recommended for analysis.

Reagent Preparation

1. Remove the kit from the refrigerator and allow it to equilibrate to room temperature for 10 minutes before use.

2. **Preparation of Positive and Negative Control Working Solutions:** Add 1 mL of Universal Diluent to each vial of lyophilized control. Let stand for 15 minutes until fully dissolved, then gently mix.

3. **Preparation of HRP-Conjugated Secondary Antibody Working Solution:** Approximately 15 minutes before use, centrifuge the 100× concentrated HRP-conjugated secondary antibody at 1,000 × g for 1 minute. Dilute it to a 1× working concentration using Universal Diluent (e.g., 10 µL concentrate + 990 µL dilution buffer). Prepare fresh before use.

4. **Preparation of 1× Wash Buffer:** Add 10 mL of 20× wash buffer to 190 mL of distilled water. Crystals may form in the concentrated wash buffer when stored at low temperature; this is normal. Allow it to reach room temperature and gently mix until the crystals are completely dissolved before dilution.

Operation Steps

1. Remove the required strips from the foil pouch after equilibrating to room temperature for 10 minutes. Reseal the remaining strips in a zip-lock bag and store at 4°C.

2. Sample Addition: Add 100 µL of sample, positive control, or negative control to the appropriate wells. Add 100 µL of Universal Diluent to the blank wells. Cover the plate with a plate sealer and incubate at 37°C for 60 minutes.

(Recommendation: Dilute test samples at least 1:1 with Universal Diluent before loading to reduce matrix effects. It is recommended to run all samples and controls in duplicate.)

3. Washing: Discard the liquid. Add 300 µL of 1× wash buffer to each well and let stand for 1 minute. Remove the wash buffer and blot dry on absorbent paper. Repeat this washing step three times (an automated washer may also be used).

4. Add HRP-Conjugated Secondary Antibody: Add 100 µL of HRP-conjugated secondary antibody working solution to each well. Cover the plate and incubate at 37°C for 30 minutes.

5. Washing: Discard the liquid and wash the plate five times according to the procedure described in Step 3.

6. Substrate Reaction: Add 90 µL of TMB substrate to each well. Cover the plate and incubate at 37°C for 15 minutes in the dark.

7. Stopping Reaction: Remove the plate and add 50 µL of stop solution to each well. Immediately measure the optical density (OD) at 450 nm.

Calculation of Results

Formula

$$S/P = \frac{\text{OD sample - mean OD negative control}}{\text{mean OD positive control - mean OD negative control}}$$

(Note: P- positive control, N-the negative control, S- the test sample)

Interpretation of Results

1. The assay is considered valid when the following criteria are met: the mean OD value of the positive control wells is > 0.20, and the mean OD value of the negative control wells is < 0.20.

2. Samples with an S/P value ≥ 0.2 are considered positive; samples with an S/P value < 0.2 are considered negative.

Kit Performance

Precision

The intra-assay coefficient of variation (CV) is $< 10\%$, and the inter-assay CV is $< 10\%$.

Troubleshooting

If the experimental results are not good, please take photos of the color development results in a timely manner, save the experimental data, keep the used plates and any unused reagents, and then contact our technical support for assistance. You may also refer to the following materials.

Problems	Possible Causes	Solutions
Linear deviation of the standard curve	Standard sample dilution is incorrect.	Ensure that the standard sample is dissolved and diluted according to the recommended method.
	Inaccurate pipetting.	Regularly calibrate the pipette and check the sealing of the tips.
	Evaporation of reaction solution.	Seal the enzyme labelling plate with sealing film.
	Incomplete washing of the plate.	Sufficient washing cycles and adequate washing liquid should be added.
	Impurities at the bottom of the wells.	Clean the bottom of the plate before reading.
Weak signal or No signal	Insufficient incubation time.	Ensure sufficient incubation time.
	Incubation temperature is incorrect.	Incubate at the recommended temperature.
	Insufficient reagent volume added.	Check the pipette and follow the operational steps rigorously.
	Incorrect dilution.	Check the reagent dilution steps.
	Enzyme conjugate inactivation.	Mix the enzyme conjugate and substrate, and check through the colour reaction.
Low OD	The microplate reader is set up incorrectly.	Check the instrument's wavelength.
	The stopping solution was not added.	Add an appropriate amount of stopping solution.
	The waiting time before reading the plate is too long.	Read the plate in a timely manner.
	The sample concentration is too high.	Determine the appropriate dilution factor through preliminary experiments.
	The sample concentration is too low.	Determine the appropriate dilution factor through preliminary experiments.
High background	The developing solution is contaminated.	Replace the developing solution.
	The developing time is too long.	Control the developing time.
	Incorrect dilution of the antibody	Use the recommended dilution method.

	or enzyme conjugate.	
	Washing of the plates is incomplete.	Ensure enough washing cycles and add sufficient washing solution.

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