Instructions for Use

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Human Anti-Single Stranded DNA Antibody (ssDNA) ELISA Kit

Catalog No.: abx364935

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 4°C for up to 6 months.

Application: For qualitative detection of ssDNA in Human Serum

Introduction: Anti-(double stranded)-DNA antibodies are highly specific markers of SLE and autoimmune (lupoid) hepatitis. Raised levels are found in 50-70% of patients with active, particularly untreated, SLE.

Principle of the Assay

This kit is based on indirect enzyme-linked immuno-sorbent assay technology. The 96-well plate is pre-coated with the target antigen. Samples are added to the wells and incubated, then washed with wash buffer. Samples that contain ssDNA will bind to the pre-coated antigen to form an antigen-antibody complex. Unbound conjugates are washed away with wash buffer. Next, HRP-conjugated detection antibody is added to the wells and incubated. TMB substrate is added, which is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The intensity of the yellow colour is proportional to the amount of ssDNA bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of ssDNA can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Positive control: 1 ml
3. Negative control: 1 ml
4. HRP-conjugated antibody: 12 ml
5. Sample diluent buffer: 12 ml
6. Concentrated Wash buffer (20X): 50 ml
7. TMB substrate A: 6 ml
8. TMB substrate B: 6 ml
9. Stop solution: 6 ml
10. Plate Sealer: 3
11. Hermetic bag: 1

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. ELISA shaker
6. Distilled or deionized water
7. 1.5 ml tubes
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.

Note:

» Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.

» Samples should be clear and transparent. Samples must be diluted so that the expected concentration falls within the kit's range.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used. Samples that contain NaN_3 cannot be detected as it interferes with HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 25 ml of concentrated wash buffer into 475 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature for at least 30 minutes prior to use.

1. Determine the number of wells to be used. Any strips that are not being used should be kept dry and stored at 4°C.
2. Set up two Negative Control wells with 100 µl of Negative Control per well. Set up two Positive Control wells with 100 µl of Positive Control per well. Set up two blank wells with no solution.
3. In a separate tube, dilute samples 1:10. Mix thoroughly. Add 100 µl of diluted samples to each sample well.
4. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 minutes.
5. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. Fill each well completely with Wash buffer (300µL) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
6. Add 100 µl of HRP-conjugate to each well (except the blank well). Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 minutes.
7. Remove the cover and discard the liquid. Wash the plate 5 times as explained in step 5.
8. Add 50 µl of TMB substrate A and 50 µl of TMB substrate B to each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 10 minutes in the dark.
9. Remove the cover and add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm within 10 minutes of adding the stop solution.

C. Analysis

1. **Calculations:**
   Mean absorbance of the positive control should be ≥ 0.5.
   Mean absorbance of the negative control should be ≤ 0.08.
   Mean absorbance of the blank wells should be ≤ 0.08.
   CUT OFF value (Negative control < 0.05) = 0.05 + 0.10 = 0.15
   CUT OFF value (Negative control ≥ 0.05) = Negative control + 0.10

2. **Interpretation of results:**
   If the postive control value is ≥ 0.5, the blank well value is ≤ 0.08, and the negative control value is ≤ 0.08, the test is valid, otherwise, the test is invalid.
   Samples:
   If O.D. of samples < CUT OFF, the test samples are considered negative.
   If O.D. of samples ≥ CUT OFF, the test samples are considered positive.
   Precision:
   Variable coefficient (CV%) ≤ 15%

C. Precautions

1. Ensure that the plate remains dry until starting the assay.
2. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
3. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all controls and samples in duplicate or triplicate.
6. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
7. Ensure plates are properly sealed or covered during incubation steps.
8. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
9. To avoid cross contamination do not reuse pipette tips and tubes.
10. The TMB Substrate B is easily contaminated; protect from light and work under sterile conditions when handling the TMB substrate solution. Equilibrate the TMB substrate at room temperature prior to use. Unreacted substrate should be colourless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.