Technical Manual

Human Anti-Double Stranded DNA (DS-DNA) Antibodies ELISA Kit

- Catalogue Code: HDES0030
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the Double Stranded DNA (DS-DNA) antibody in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified DS-DNA antigen. Samples are added to the ELISA Microtiter plate wells and the DS-DNA -Ab in which will combine with the pre-coated antigen to form antigen-antibody compound. Free components are washed away. The HRP Conjugate is added to each well and react with the compound to form “DS-DNA antigen- DS-DNA antibody-HRP conjugate” compound. The TMB substrate is added to initiate the color developing reaction. The presence of DS-DNA -Ab can be determined according to the OD value after colorimetric assay with the Micro-plate reader.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Positive Control	0.5 mL
Negative Control	0.5 mL
HRP Conjugate	12 mL
Sample Diluent	12 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

3. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper
- Loading slot for Wash Buffer

4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0030. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0030 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

5. Storage and expiry date

Store unopened at 2-8° C. Do not freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 1 month.
Expiry date: expiration date is on the box.

6. Sample preparation

1. **Serum:** Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test.
2. Samples with sodium azide cannot be detected. Because the sodium azide may inhibit the HRP activity. The suspended fibrous protein may cause a false positive if not fully precipitated. Avoid of samples with hyperlipidemia, hemolysis or jaundice. Obviously contaminated samples can't be detected.
3. Samples can be stored at 2-8° C for one week. If samples not tested in a week, store them below -20° C and avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
4. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and control in order (multiple well), and keep a record of control wells and sample wells. Set 1 well for blank control (empty), 3 wells for negative control and 1 well for positive control. **Samples need test in duplicate** (Blank well is not necessary for dual-wavelength detection).
2. **Add sample:**
   (1) Add 100 μL of **negative control**/**positive control** respectively to 3 negative control wells, 1 positive control well, keep the blank control well empty.
   (2) Dilute the tested **Serum** with **Sample Diluent** at 1:10 into sample well (add 100 μL of Sample Diluent and add 10 μL of serum sample), mix fully.
3. **Incubate:** gently tap the plate to mix thoroughly, cover the ELISA Microtiter plate with plate sealer. Incubate for 30 minutes at 37° C in shading light.
4. **Wash:** after incubation, remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30 s each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 100 μL of **HRP Conjugate Working Solution** to each well except the blank control well.
6. **Incubate:** cover the ELISA Microtiter plate with plate sealer. Incubate for 30 minutes at 37° C in shading light.
7. **Wash:** repeat step 4.
8. **Add substrate:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to ensure thorough mixing. Cover with a new plate sealer. Incubate for 10 minutes at 37° C in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min.**

8. Reference value

Normally, blank well (just chromogenic agent and stop solution) absorbance: $A_{450} \leq 0.08$; positive control (PC): $A_{450} > 0.50$ and average A value of negative control (NC): $A_{450} < 0.08$.

9. Interpretation of the results

Calculate the Cut Off: Cut Off (C.0) = 0.10 + negative control (NC) average A value (when NC average A_{450} < 0.05, calculate at 0.05; while NC average A_{450} ≥ 0.05, calculate at the actual value).

(1) Positive result: Sample absorbance ≥ Cut Off.

(2) Negative result: Sample absorbance < Cut Off.

10. Interpretation of results

1. Negative result indicates there is no DS-DNA antibody detected in samples, while positive result means the opposite.
2. The positive result of DS-DNA antibody is an important auxiliary index of diagnosis of SLE.

11. Limitations of test method

1. This test is only used as the qualitative detection of DS-DNA antibody in serum of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com