Technical Manual

Human anti-Cyclic Citrullinated Peptide (CCP) Antibodies ELISA Kit

- Catalogue Code: HDES0025
- Antibody ELISA Kit
- Research Use Only

1. Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the anti-Cyclic Citrullinated Peptide (CCP) antibody in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with purified CCP antigen. Samples are added to the ELISA Microtiter plate wells and the CCP -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 “CCP antigen- CCP antibody-HRP conjugate” compound. The TMB substrate is added to initiate the color developing reaction. The presence of CCP -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. Experimental instrument

- Micro-plate 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 HDES0025. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0025 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 at 2-8° C.Avoid 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 packing box.

6. Sample preparation

1. Serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for test. Anticoagulant (such as EDTA, sodium citrate and heparin, etc.) in samples do not affect the results.
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 or stored at -20 ° C for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.

7. Assay procedure

Bring all reagents to room temperature for 30 min. Dilute the 20×Concentrated Wash Buffer for 20 times with distilled water.

1. **Add sample:**
   (1) Reserve 1 well for blank control (empty), 3 wells for negative control, 1 well for positive

control (100 μL control serum for each well). (Blank well is not necessary for dual-wavelength detection)

(2) Dilute the tested serum with Sample Diluent at 1:10 (add 100 μL of Sample Diluent to the reaction well, and then add 10 μL of serum sample), mix fully.

(3) Gently tap the plate to mix thoroughly.

2. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
3. **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 30-60 sec each time.
4. **HRP conjugate:** Add 100 μL of HRP Conjugate working solution to each well except the blank control well.
5. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min at 37° C.
6. **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 30-60 sec each time.
7. **Add substrate:** Add 50 μL of Substrate Reagent A and 50 μL of Substrate Reagent B to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 10 min at 37° C in dark (The reaction time can be extended according to the actual color change).
8. **Stop reaction:** Add 50 μL of Stop Solution to each well, gently tap the plate to mix thoroughly.
9. **OD Measurement:** Set the Micro-plate 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 needed when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 10 min.**

8. Reference value

1. **Result analysis**

(1) Use each test result independently. Determine the result according to the Cut Off value.

(2) Calculate the Cut Off: Cut Off(C.0) = 0.10 + A value of average negative control (NC) (when A450 of average NC $<$ 0.05, calculate at 0.05; while A450 of average NC $\geqslant$ 0.05, calculate at the actual value).

2. **Quality control**

(1) Blank well (just chromogenic agent and stop solution): A450 $\leqslant$ 0.08.

(2) Positive control (PC): A450 $>$ 0.50.

(3) Negative control (NC): A450 $<$ 0.08.

The experimental result is valid if quality control is valid.

3. **Determination of results**

(1) Positive result: A450 of Sample ⩾ Cut Off.
(2) Negative result: A450 of Sample ＜ Cut Off.

9. Interpretation of results

1. Negative result indicates there is no CCP antibody detected in samples, while positive result means the opposite.
2. The positive result of CCP antibody is an important auxiliary index of diagnosis of chronic infectious arthritis.

10. Limitations of test method

1. All high sensitivity immune experiment system exists potential non-specificity. Therefore, unacceptable positive results may be caused by biological false positive of ELISA method.
2. Any positive result should be determined combined with clinical information.
3. This kit is used for qualitative detection of human serum only, and the detection results in other samples are still unclear.

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