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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !)

Human Anti-Helicobacter Pylori (HP) IgG ELISA Kit

Catalog No: E-HD-E127

96T/96T*2

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This ELISA kit uses Indirect-ELISA as the method to detect the Helicobacter Pylori (HP) antibody in human serum. The ELISA Microtiter plate is pre-coated with recombinant Cag-A(I) and Hsp-58(II) antigen. When samples are added into the ELISA Microtiter plate wells, the HP-Ab in the samples will react with the pre-coated HP antigen to form antigen-antibody compound. Free components will be washed away. HRP conjugated Mouse-anti-human IgG is added to each well. Free components will be washed away. The TMB substrate solution is added to initiate the color developing reaction. The shade of developed color is proportional to the concentration of HP-Ab.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
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
Positive Control	1 mL
Negative Control	1 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other materials required but not supplied

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

Notes

1. This kit is for research use only. It is disposable and cannot be used repeatedly.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. 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.
4. 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.
5. 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.
6. Concentrated Wash Buffer at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
7. Each well must be filled with liquid when washing in order to prevent residual free enzyme.
8. The results shall depend on the readings of the micro-plate Reader.
9. **Each reagent is optimized for use in the E-HD-E127. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E127 with different lot numbers.**
10. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

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.

Sample preparation

1. **Serum:** Fresh collected serum specimens should be fully centrifuged, then take clear liquid for test, or suspended fibrous protein may cause a false positive. Samples can be stored at 2-8°C for 24 h or at -20°C for long-term storage. Avoid repeated freezing and thawing.
2. Avoid using samples with hemolysis or lipidemia. Don't use polluted samples.
3. 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.

Assay procedure

Restore all reagents and samples to room temperature (25°C) for 30 min 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, 2 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 **Positive/Negative Control** respectively to **Positive/Negative Control** wells, keep the blank control well empty.
 - (2) Dilute the tested **Serum** with **Sample Diluent** at 1:20 into sample well (add 100 µL of sample diluent and add 5 µL of sample), mix fully.
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA plate with sealer. Incubate for 30 min at 37°C in shading light.
4. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 300 µL of wash buffer to each well and immerse for 30 s each time. Repeat wash procedure for 5 times, 30 sec intervals/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** to each well except the blank control well.
6. **Incubate:** Cover the ELISA plate with sealer. Incubate for 30 min 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 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).
9. **Stop reaction:** Add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
10. **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. This step should be finished in 10 min after stop reaction.

Reference value

Normally, blank well (just chromogenic agent and stop solution) absorbance: $A_{450} \leq 0.08$.

Positive control (PC): $A_{450} > 0.30$; Negative control (NC): $A_{450} < 0.08$.

Interpretation of test results

Cut Off (C.O) = $0.10 + \text{average A value of negative control (NC)}$ (when average A_{450} of NC < 0.05 , calculate at 0.05; while average A_{450} of NC ≥ 0.05 , calculate at the actual value).

1. Positive result: A_{450} of Sample \geq Cut Off.
2. Negative result: A_{450} of Sample $<$ Cut Off.
3. Negative result indicates there is no HP antibodies detected in samples, while positive result means the opposite.

Limitations of test method

1. This test is only used as the qualitative detection of HP antibodies 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.