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

Thyroid Stimulating Hormone ELISA Kit

Catalog No: E-HD-E172

96T

Version Number:	V1.0
Revision Date:	2024.10.15

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

Enzyme-linked immunoassay was used. Two monoclonal antibody strains were used, one was used for solid-phase coating to prepare solid phase antibody, and the other was used for HRP labeling to prepare HRP- antibody. TSH (standard or test sample) and HRP-antibody were added to the micropores coated with antibodies, and the "solid phase antibody-antigen-HRP-antibody" complex was formed after equilibrium. With the increase of TSH concentration, the A value (or absorbance, OD value) of color rendering gradually increased in a linear relationship.

Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1 mL each (0、0.3、0.6、1.5、3.0、6.0、12.0mIU/L)
HRP Conjugate	6 mL
Quality Control Solution	1 mL*2 Level 1 is (1.51~2.81mIU/L), Level 2 is (4.80~8.92mIU/L)
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 mL
20×Concentrated Wash Buffer	15 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

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℃ Incubator or water bath

Deionized or distilled water

Absorbent paper

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 E-HD-E172. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-HD-E172 with different lot numbers.**
8. 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℃. Avoid freeze.

Please store the opened plate at 2-8℃, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the packing box.

Sample preparation

1. The **serum** was separated after the blood sample was taken and stored at 2-8 ℃ for no more than 48 hours, and at -15℃ for 3 months.
2. Anticoagulant (EDTA, sodium citrate and heparin sodium) in samples do not affect the result of the experiment in general. Avoid of samples with suspended fibrous protein, aggregation or severe hemolysis (hemoglobin > 2 g/L), hyperlipemia (triglyceride > 4mmol/L), high bilirubin (bilirubin > 150 μmol/L), cholesterol (cholesterin > 6mmol/L).
3. **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℃) 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℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.** Set 1 well for blank control.
2. **Add sample:** add 100 μL of **Standard Liquid** per well (Set two holes for each calibration point in sequence), then add 100 μL of **Quality Control Solution** or **test serum** directly to each of the remaining detection wells. Then add 50μL of **HRP Conjugate** to each well, mix thoroughly, apply a sealing plate membrane, and incubate at 37 ℃ for 2 hours.
3. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 3 times with **Wash Buffer** and immerse for 30-60 sec 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).
4. **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 15 min at 37℃ in shading light.
5. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
6. **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.

Result analysis

1. Dual-wavelength enzyme labeling instrument can be set without blank control hole, and no zero adjustment is required. A single wavelength enzyme-labeled instrument must be set with a blank control hole, which is first zeroed and then measured.
2. Mapping method: Take the absorption value of calibration product S1 ~ S5 as the vertical axis (log coordinate), and the corresponding concentration as the horizontal axis (log logarithmic coordinate), draw the calibration curve on the logarithmic coordinate paper, and find the content of the specimen to be measured on the calibration curve.
3. Computer: The concentration is calculated by computer.

Limitations of test method

1. Severe hemolysis and chyle blood may affect the test results.
2. The sample tested for this product with a concentration of 120mIU/L did not exhibit a "Hook Effect".