Technical Manual

Human Free Thyroxine ELISA Kit

- **Catalogue Code: HDES0102**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses Competition-ELISA as the method to detect the Free Thyroxine (FT_4) in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with anti-T_4 synthetic analogue antibody. Add samples, biotin labeled T_4 synthetic analogue and HRP-streptavidin conjugate in sequence. If FT_4 exist in sample will competition with biotin labeled T_4 synthetic analogue, finally form a anti-T_4 synthetic analogue antibody, biotin labeled T_4 synthetic analogue and HRP-streptavidin conjugate complex. Free components are washed away. The substrate reagent is added to initiate the color developing reaction. The presence of T_4 can be determined according to the absorbance value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	1.0 mL each (0 pmol/L, 4 pmol/L, 8 pmol/L, 16 pmol/L, 32 pmol/L, 64 pmol/L)
Biotinylated Antigen	6 mL
20×Concentrated Wash Buffer	15 mL
HRP conjugate	6 mL
Quality Control (2 level)	1.0 mL×2 (2 level)
Substrate Reagent A	7 mL
Substrate Reagent B	7 mL
Stop Solution	7 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.

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

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 HDES0102. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0102 with different lot numbers.**
8. Different batch numbers of reagents should not be mixed.
9. 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:** Human serum can be used as detected sample. Obviously contaminated samples can't be detected. Samples can be stored at 2-8° C for 48 h and stored at -15° C for 3 months. Avoid freeze-thaw cycle above 3 times. Freezing samples should be mixed fully before test.
2. Avoid of using hemolysis, turbidity, or lipid blood samples.
3. **Wash Buffer:** The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) 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 standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 µL of **Standard, Sample** or **Quality Control** per well, set the blank control well. Then add 50 µL of **Biotinylated Antigen**, (except for blank control well)..
3. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 60 min at 37° C.
4. **Wash:** remove the plate sealer and aspirate the liquid of each well. Immediately fill each well with Wash Buffer and wash. Repeat the washing procedure for 3 times and immerse for 10 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).
5. **HRP Conjugate:** add **HRP Conjugate** into each well (except for blank control well).
6. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37° C.
7. 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 15 min at 37° C in shading light.
9. **Stop reaction:** add 50 µL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
10. **OD Measurement:** set the Microplate Reader wavelength at 450 nm to detect A value of each well, zero set with blank well (or not set blank well, set the Microplate Reader dual-wavelength at 450/630 nm to detect A value of each well).

8. Result analysis

1. The dual wavelength Microplate Reader does not require a blank control well or zero point adjustment. A single wavelength Microplate Reader must set a blank control well. First, zero set the blank control well, and then measure. If there is no zero set, the A value of each well should subtracted the A value of the blank control well.
2. Create a standard curve by plotting the absorbance of each standard on the y-axis against the concentration on the x-axis, and set double log fit to draw a standard curve. Add average absorbance value of sample to standard curve to get corresponding concentration.

9. Reference value

Normal reference: 8.4-26.8 pmol/L, average 17.60±4.40 pmol/L.
Due to differences in geography, race, gender and age, it is recommended that each laboratory establish its own reference value (range).

10. Product performance

1. **Accuracy:** Prepare the national standard substance with S_0 standard to a concentration of 60.8 pmol/L, analyze and determine it using the standard soluton in the kit, and fit with log-log. The ratio of the measured titer to the labeled titer of the national standard substance should be in the range of 0.90~1.10.
2. **Limit of detection:** should not be more than 4.0 pmol/L.
3. **Linear range:** in the range of [4, 64] pmol/L, the correlation coefficient r≥0.990.
4. **Repeatability:** CV ≤ 15%.
5. **Inter-batch difference：** CV ≤ 15%.
6. **Quality control measurement value:** Each test result should be within the allowable range.
7. **Specificity:** There was no significant cross-reaction with triiodothyronine (T_3). Prepared high concentrations of T_3 solutions, and the measurement results should conform to the table below.

Substance	Sample concentration	Measured value
T_3	50 ng/L	≤ 2.0 pmol/L

11. Limitations of this test method

1. This product can only be used for the determination of serum samples and can not be used for other body fluid samples.
2. To avoid interfering with the results, serum with high blood lipids and hemolysis should be avoided. Samples with obvious contamination should not be used for testing as they may lead to erroneous experimental results.

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