

Free Thyroxine (FT4) ELISA Test Kit

NAME AND INTENDED USE

Detection Kit for Free Thyroxine (FT4) (Enzyme-Linked ImmunoSorbent Assay, ELISA) It is used in quantitative tests for Free Thyroxine (FT4) in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Human thyroid hormones (triiodothyronine (T3), thyroxine (T4)) are mostly associated with plasma proteins (thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), serum white Protein (Alb)). Only about 0.3% of the body's thyroid hormone have specific physiological function. Therefore, the determination of free triiodothyronine (FT3), free Thyroxine(FT4) concentration can reflect the function and status of the thyroid. It can avoid misdiagnosis caused by the changes of TBG concentration.

PRINCIPLE

FT4 kit is based on competitive method. Coated micro-well plates with anti-T4 to form solid-phase antibody. The FT4 of sample and biotin-T4-X compete with anti-T4 on solid-phase. Equilibrium, avidin-HRP added, [solid phase antibody]-[biotinylated FT4]-[avidin-HRP] complex was formed. Add substrate and detect absorbent value. Calculate FT4 content of patient serum through computer or plotting fitting concentration-absorbent value curve.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the FT4 procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens can be stored up to 48 hours at 2-8°C. Specimens held for a longer time can be frozen at -20°C and avoid repeated freeze melting.

Serum samples with concentrations expected to be greater than 64 pmol/L should be diluted with normal saline.

NOTE: If needed, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8x12 wells), Ready to use. Coated with anti-T4 antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2-8°C until expiration date.
2. Biotin-FT4: 1 vial of 6ml, Ready to use. Store at 2-8°C until expiration date.
3. Avidin-HRP Conjugate: 1 vial of 6ml, Ready to use. Store at 2-8°C until expiration date.
4. Calibrator: 6 vials of 1ml, Ready to use. Labeled with S0 to S5 and the concentration of T4 is 0, 4, 8, 16, 32, 64pmol/L. Store at 2-8°C until expiration date.
5. Control: 2 vials of 1ml, Ready to use. Labeled with high value and low value. Store at 2-8°C until expiration date. The concentration of low value is 8.4-15.6pmol/L. The concentration of high value is 22.4-41.6pmol/L.
6. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
7. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.
8. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2-8°C until expiration date.

9. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2-8°C until expiration date.
10. Plate sealer: 2 pieces.
11. Plastic resealable bag: 1 set.
12. Instruction manual: 1 copy.

RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 3 times with about 0.3ml wash buffer. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all wash buffer out and block the rim of well on absorbent paper for a few seconds. Filling each well with water and remain 10 seconds. Repeat these steps 3 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.
3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Read procedure. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
5. Storage. The whole kit should be stored at 2-8°C for one year. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.
6. Control serum is prepared with human serum, which is tested negative of HBV, HCV and HIV. But it should still be considered as capable of transmitting viral diseases.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash Solution by diluting Wash Concentrate 20-fold with deionized water. The diluted wash solution is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. All the Calibrator and controls should set duplicate.
2. Dispense 50 µl of calibrator/controls/samples into wells.
3. Dispense 50 µl of Biotin-FT4 to each well.
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 1 hour.
5. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
6. Dispense 50 µl of avidin-HRP Conjugate to each well.
7. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 30 minutes.
8. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
9. Dispense 50 µl of chromogen A to each well.
10. Dispense 50 µl of chromogen B to each well.
11. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
12. Dispense 50 µl of stop solution to each well and mix completely.
13. Read the absorbance of the plate within 10 minutes. (See read procedure)

CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation: $\log OD = B * \log [\text{concentration}] + A$

PERFORMANCE CHARACTERISTICS

1. Expected value
The cut-off value of FT3 concentration for healthy subjects is 8.4-26.8 pmol/L.
2. Sensitivity
The detection limit of the assay is approximately 4 pmol/L.
3. Precision CV < 15%
4. Specificity: No cross reactions with T3.

CODIGO:RSET103-3