Free Thyroxine (fT4) ELISA Kit

Cat #: orb1173297 (manual)

Size: 48 T/96 T

Product name: Free Thyroxine (fT4) ELISA Kit

Catalog number: orb1173297

Detection range: 4 pmol/L- 64 pmol/L

Sensitivity: 4 pmol/L

Precision: Intra-assay Precision: The CV (%) < 15%. Inter-assay Precision :The CV (%) < 15%

Recovery: The recovery ranged from 85% to 115% with an overall mean recovery of 100%.

Specificity: Free Thyroxine (fT4) ELISA Kit has high sensitivity and excellent specificity for detection of fT4. No significant cross-reactivity or interference between fT4 and analogues was observed.

Applicable samples: Serum, Plasma

Storage: The unopened kit should be stored at 4℃ for 12 months.

Assay Principle

Over 99% of Triiodothyronine (T4) circulates in blood is bound to carrier proteins; thyroxine-binding globulin (TBG). However, only the free (unbound) portion of T4 is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total T4 level changes so that the free T4 concentration remains constant. Thus, measurements of free T4 concentrations correlate more reliably with clinical status than total T4 levels. The increase in total T4 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T4 levels while the free T4 concentration remains basically unchanged. Free Thyroxine (fT4) ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to fT4. Standards or samples are added to the appropriate microtiter plate wells with Biotin-conjugated fT4. A competitive inhibition reaction is launched between fT4 (Standards or samples) and Biotin-conjugated fT4 with the pre-coated antibody specific for fT4. The more amount of fT4 in samples, the less antibody bound by Biotin-conjugated fT4. After washing, Avidin-HRP is added to the wells. Substrate solution is added to the wells and the color develops in opposite to the amount of fT4 in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
fT4 Standard	0.5 mL×5	1 mL×5	4℃
Avidin-HRP	3 mL	6 mL	4℃

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Biotin Conjugated fT4	3 mL	6 mL	4℃
HRP Substrate A	3.5 mL	7 mL	4℃, protected from light
HRP Substrate B	3.5 mL	7 mL	4℃, protected from light
Stop Solution	3.5 mL	7 mL	4℃
Wash Buffer (20×)	7.5 mL	15 mL	4℃
fT4 Microplate	48 wells	96 wells	4℃
Plate Covers	1	2	RT

Note: Std1: 4 pmol/L; Std2: 8 pmol/L; Std3: 16 pmol/L; Std4: 32 pmol/L; Std5: 64 pmol/L.

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 450 nm
- Multi-channel pipette or automated microplate washer
- Incubator, refrigerated centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

1×Wash Buffer: Wash Buffer (20×) dilute with deionized water 1:20 to obtain the 1×Wash Buffer. Store at 4℃.

Sample Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
2. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens. If samples are to be used within 24 hours, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

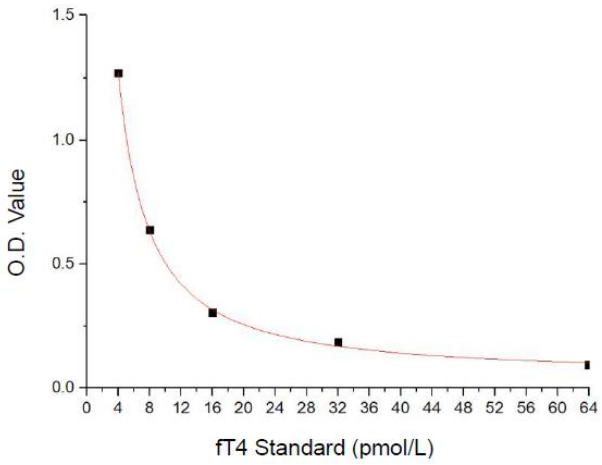
2. Add 50 µL of fT4 Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.
3. Add 50 µL of Biotin Conjugated fT4 to each well (not to Blank well). Mix well, cover with the plate cover provided and then incubate for 1 h at 37℃.
4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 µL) using a Multi channel pipette or automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
5. Add 50 µL of Avidin-HRP to each well (not to Blank well), mix well and cover with the plate cover provided. Incubate for 30 min at 37℃.
6. Repeat the wash as in step 4.
7. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.
8. Add 50 µL of Stop Solution to each well. Stop Solution should be added to the plate in the same order as HRP Substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and sample.
2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean absorbance for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve（$R^2 \geq 0.99$）



Standard Curve of fT4 in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.
4. Stop Solution has certain Corrosive. Please take protective measures when operating.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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