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Product Information

Free Thyroxine (fT_4) ELISA

Catalog Number **SE120122**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Over 99% of thyroxine (T_4) circulates in blood is bound to carrier proteins; Thyroxine-Binding Globulin (TBG). However, only the free (unbound) portion of thyroxine 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 T_4 level changes so that the free T_4 concentration remains constant. Thus, measurements of free T_4 concentrations correlate more reliably with clinical status than total T_4 levels. The increase in total T_4 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T_4 levels while the free T_4 concentration remains basically unchanged.

The Free Thyroxine (fT_4) ELISA is used for the quantitative measurement of free Thyroxine (fT_4) in human serum. It is a solid phase competitive ELISA. The samples Anti-T_4 Biotin and fT_4 enzyme conjugate are added to the wells coated with streptavidin. fT_4 in the serum competes with a T_4 enzyme conjugate for binding sites. Unbound T_4 and T_4 Enzyme Conjugate are washed off. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of fT_4 in the samples. A standard curve is prepared relating color intensity to the concentration of the fT_4.

Components

Materials Provided	96 Tests
Microwells coated with Streptavidin	12 x 8 x 1
fT_4 Standard: 6 vials (ready to use)	0.5 mL
Anti-T_4 Biotin Solution	7 mL
fT_4 Enzyme conjugate: 1 Bottle (ready to use)	7 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2–8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (–20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

20x Wash Buffer Concentrate

Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun, or strong light.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that standards, control and serum samples be run in duplicate.

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Format the microplates wells for control, standard, and samples to be assayed in duplicate. Place any unused microwell strips back into the aluminum bag, seal, and store at 2–8 °C.
2. Pipette 25 µL of fT_4 standards, control, and samples into the assigned well.
3. Add 50 µL of fT_4 enzyme conjugate to all wells.
4. Add 50 µL of Anti-T_4 Biotin Solution to all the wells.
5. Incubate for 60 minutes at room temperature (18–26 °C).
6. Remove liquid from all wells. Fill wells with 300 µL of 1x Wash buffer Wash three times. Blot on absorbent paper towels.
7. Add 100 µL of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µL of Stop Solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

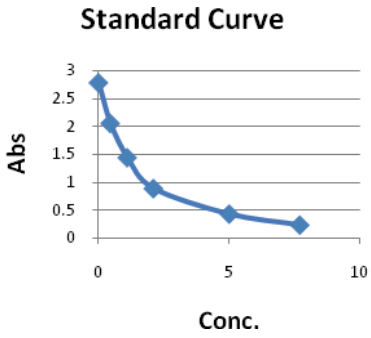
Results

The standard curve is constructed as follows:

1. Check fT_4 standard value on each standard vial. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. To construct the standard curve, plot the absorbance for fT_4 standards (vertical axis) versus fT_4 standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of standard curve

	OD 450 nm	Concentration (ng/dL)
Std 1	2.786	0
Std 2	2.056	0.45
Std 3	1.440	1.10
Std 4	0.885	2.10
Std 5	0.426	5.00
Std 6	0.229	7.70



Expected values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for fT_4 were established and may be used as initial guideline ranges only:

Adult: 0.8–2.0 ng/dL

Note: The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings, and other diagnostic procedures.

References

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