PicoKine™ ELISA

Catalog number: EK7054

For the quantitation of **Human Free Thyroxine (FT4)** concentrations in Serum.

This package insert must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

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Human Free Thyroxine (FT4) ELISA Kit (Competitive ELISA)

Catalog Number: EK7054

Introduction

This Bosterbio ELISA kit is designed to measure the concentration of FT4 in human serum by Competitive ELISA (Enzyme-Linked Immunosorbent Assay). The polystyrene micro-well plate in this kit has been pre-coated with an anti-Human FT4 antibody. Sample or standards are added to the wells along with a fixed quantity of biotinylated FT4 and incubated. The FT4 found in the sample or standards competes with the biotinylated FT4 for limited binding sites on the immobilized anti-Human FT4 antibody. Excess unbound biotinylated FT4 and sample or standard FT4 is washed from the plate. Avidin-HRP conjugate is added, incubated and washed. An enzymatic reaction is then produced through the addition of substrate which is catalyzed by the immobilized HRP to generate a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration is measured by reading the absorbance at 450 nm which is quantitatively proportional to the amount of biotinylated FT4 captured in the well and inversely proportional to the amount of FT4 which was contained in the sample or standard.

Overview

Product Name	Human Free Thyroxine (FT4) ELISA Kit (Competitive ELISA)
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Competitive High Sensitivity ELISA kit for Quantitative Detection of Human FT4. 96wells/kit, with removable strips.
Sensitivity	4.0 pmol/L *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	4-64 pmol/L
Storage Instructions	Store the kit at 2°C to 8°C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose reagent to heat, sun, or strong light. Avoid multiple freeze-thaw cycles(Shipped with wet ice.)
Specificity	Natural and recombinant Human FT4
Cross Reactivity	There is no detectable cross-reactivity.

Kit Components/Materials Provided

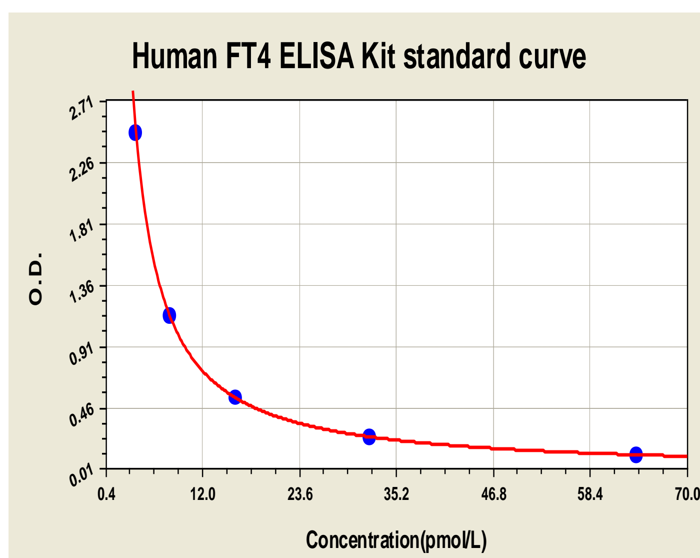
Description	Quantity	Volume	Buffers
Pre-coated 96-well strip microplate	1	8 strips of 12 wells	Rabbit Anti- FT4 Antibody, Polystyrene micro-well plate
Human FT4 Standards(S0~S5)	6	1ml	FT4 (0, 4, 8, 16, 32, 64 pmol/L), 0.02M PBS, 50 % detoxification serum, 0.1% Proclin-300
Avidin-HRP conjugate	1	6ml	Avidin-HRP conjugate, 0.02M PBS, 20% new-born calf serum, 0.01% azophloxine, 0.1% Proclin-300
Biotinylated antigen	1	6ml	Biotinylated FT4 analogue, 0.02M PBS, 20% new-born calf serum, 0.1% Proclin-300
Controls	2	1ml	FT4, 100% natural protein, 0.1% Proclin-300
20X Wash Buffer Concentrate	1	15ml	0.2M PBS containing 0.5% tween 20
Color Developing Reagent A	1	7ml	11m mol/L Urea hydrogen peroxide
Color Developing Reagent B	1	7ml	2m mol/L 3,3'5,5'-Tetramethylbenzidine
Stop Solution	1	7ml	2mol/L Sulphuric acid
Plate Sealers	2	Piece	

Required Materials That Are Not Supplied

1. Microplate Reader capable of reading absorbance at 450nm.
2. Automated plate washer (optional)
3. Pipettes and pipette tips capable of precisely dispensing 0.5 μl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
4. Deionized or distilled water.
5. 500ml graduated cylinders.
6. Test tubes for dilution.

Typical Data Obtained from Human FT4 ELISA Kit

Concentration(pmol/L)	0	4	8	16	32	64
O.D.	0.000	2.476	1.139	0.534	0.245	0.117



Warnings and Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Sample Preparation and Storage

1. Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
2. Avoid multiple freeze-thaw cycles.
3. Prior to assay, frozen sera should be completely thawed and mixed well.
   Note: Grossly hemolyzed samples and chylemia samples are not suitable for use in this assay, so the samples should be centrifugated adequately and no hemolysis or granule was allowed.

Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (20-25°C) for 30 minutes.
20X Wash Buffer Concentrate	Prepare 1X wash buffer by adding 15 ml of Wash Buffer Concentrate to 285 ml deionized or distilled water to prepare 300 mL of Wash Buffer.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Set Standard wells, Sample wells, Control wells and Blank wells, add 50 µl of the standard, sample, or control per well. At least two replicates of each standard, sample, control or blank is recommended.
4. Then add 50 µl of Biotinylated antigen to each well except for the blank well and mix thoroughly.
5. Cover with plate sealer and incubate for 60 minutes at 37°C.
6. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
7. Add 50 µl Avidin-HRP conjugate to each well except for the blank well and mix thoroughly.
8. Cover with plate sealer and incubate for 30 minutes at 37°C.
9. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
10. Add 50µl Color Developing Reagent A and 50µl Color Developing Reagent B to each well and incubate in the dark for 15 minutes at 37°C.
11. Add 50 µl of Stop Solution to each well.
12. Read absorbance on Plate Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average blank O.D. reading.

It is unnecessary to set blank control for dual wavelength plate reader.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic- curve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

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