



EAGLE
BIOSCIENCES

Free Thyroxine (fT4) ELISA Kit

Catalog Number:
T4F31-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 8.0 (effective 12JAN24)

EAGLE BIOSCIENCES, INC.

20A Northwest Blvd., Suite 112, Nashua, NH 03063

Phone: 617-419-2019 Fax: 617-419-1110

WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Free Thyroxine (FT4) ELISA Kit (enzyme-linked immunoassay kit) is intended for the quantitative measurement of free Thyroxine (fT4) in human serum. The Eagle Biosciences Free Thyroxine (FT4) ELISA Kit is for research use only and not to be used in diagnostic procedures.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

PRINCIPLE OF THE ASSAY

This Eagle Biosciences Free Thyroxine (FT4) ELISA Kit is a competitive immunoassay. Competition occurs between fT4 present in standards, controls, specimen samples and an enzyme labelled antigen (HRP conjugate) for a limited number of anti-T4 antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-colored product that is inversely proportional to the amount of fT4 present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the color from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of standards used to plot a standard curve from which the amount of fT4 in specimens samples and controls can be directly read.

The labelled T4 (HRP conjugate) employed in this assay system has shown no substantial binding properties towards thyroxine binding globulin (TBG) or human serum albumin (HSA). The binding sites on the microplates are designed to be of a low binding-capacity in order not to disturb the equilibrium between T4 and its carrying proteins. The assay is carried out under normal physiological conditions of pH, temperature and ionic strength.

PROCEDURAL WARNINGS AND PRECAUTIONS

- This kit is for use by trained laboratory personnel (professional use only). For research use only.
- Practice good laboratory practices when handling kit reagents and specimens. This includes:
- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
- Wear protective clothing and disposable gloves.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes, use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Do not use this kit beyond the expiry date stated on the label.
- If the kit reagents are visibly damaged, do not use the test kit.
- Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
- All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
- A standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or sample pools which should be included in every run at a high and low level for assessing the reliability of results.

- The controls (if applicable with this kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper reagent storage.
- When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
- The TMB Substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
- Avoid microbial contamination of reagents.
- To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard, and control.
- To prevent contamination of reagents, do not pour reagents back into the original containers.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
- Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
- This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
- The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
- Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
- If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shake and/or speed is used, the user is responsible for validating the performance of the kit.
- Do not reuse the microplate wells, they are for SINGLE USE only.
- To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the participant is established.
- When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

SAFETY CAUTIONS AND WARNINGS

BIOHAZARDS

The reagents should be considered a potential biohazard and handled with the same precautions applied to human specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

SPECIMEN COLLECTION, STORAGE, AND PRE-TREATMENT

Specimen Collection & Storage

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 mL of venous blood into an appropriately labeled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage tube or container. Serum samples may be stored at 2-8°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Specimen Pre-Treatment

Specimen pre-treatment is not required.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Calibrated single-channel pipette to dispense 25 µL.
- Calibrated multi-channel pipette to dispense 50 µL, 100 µL, and 150 µL
- Calibrated multi-channel pipettes to dispense 300 µL (if washing manually)
- Automatic microplate washer (recommended)
- Disposable pipette tips
- Distilled or deionized water
- Calibrated absorbance microplate reader with a 450 nm and an upper OD limit of 3.0 or greater
- A 37°C incubator

REAGENTS PROVIDED

1. Microplate

Contents: One anti-T4 polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format: Ready to Use
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

2. HRP-Conjugate Concentrate (51x)

Contents: One bottle containing T4-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format: Concentrated; Requires Preparation
Volume: 0.3 mL/bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Preparation: Discard after use.
Preparation: **Dilute 1:51** in assay buffer before use (e.g., 40 µL of conjugate concentrate in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of conjugate concentrate in 12 mL of assay buffer. Discard any that is left over.

3. Standard A - E

Contents: Five bottles of standard containing specified free T4 concentrations. Human serum-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of T4.

Listed below are approximate concentration, please refer to vial labels for exact concentrations.
Concentrations: 0, 2, 6, 20, 80 pg/mL.

Format: Ready to Use

Volume: 0.5 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

4. **Control 1 - 2**

Contents: Two bottles of control containing different free T4 concentrations. Human serum-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of free T4.

Refer to the QC certificate for the target values and acceptable ranges.

Format: Ready to Use

Volume: 0.5 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

5. **Assay Buffer**

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.

Format: Ready to Use

Volume: 15 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

6. **TMB Substrate**

Contents: One bottle containing a tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Format: Ready to Use

Volume: 16 mL/bottle

Storage: 2-8°C

Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks.

7. **Stopping Solution**

Contents: One bottle containing 1M sulfuric acid.

Format: Ready to Use

Volume: 6 mL/bottle

Storage: 2-8°C

Stability Unopened: Stable until the expiry date printed on the label.
After Opening: Stable for four weeks

8. Wash Buffer Concentrate (10x)

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative
Format: Concentrated; Requires Preparation
Volume: 50 mL/ bottle
Storage: 2-8°C
Stability: Unopened: Stable until the expiry date printed on the label.

After Opening: Stable for four weeks. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.

Preparation: **Dilute 1:10** in distilled or deionized water before use. If the whole microplate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of distilled or deionized water.

RECOMMENDED ASSAY LAYOUT*

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 1	STD 1	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
B	STD 2	STD 2	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	STD 3	STD 3	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
D	STD 4	STD 4	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
E	STD 5	STD 5	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
F	STD 6	STD 6	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
G	control 1	control 1	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
H	control 2	control 2	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample

**Layout subject to change based on standard and control quantities*

ASSAY PROCEDURE

All kit components, controls, and specimen samples must reach room temperature prior to use. Standards, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.

2. Prepare the HRP Conjugate Working Solution and Wash Buffer Working Solution (see Reagents Provided).
3. Plan the microplate wells to be used for standards, controls, and samples. Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.
4. **Pipette 25 µL** of each standard, control and specimen sample into assigned wells.
5. **Pipette 100 µL** of the HRP Conjugate Working solution into each well (the use of a multichannel pipette is recommended.)
6. **Gently tap** the microplate frame for **10 seconds** to mix the contents of the wells.
7. **Incubate** the microplate inside a **37°C incubator** for **60 minutes**.
8. Wash the microplate wells with an automatic microplate washer (preferred) or manually as stated below.
 - a. Automatic: Using an automatic microplate washer, perform a 3-cycle wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells then filling each well with 300 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.
 - b. Manually: Using an automatic microplate washer, perform a 3-cycle wash using **300 µL/well** of Wash Buffer Working Solution (3 x 300 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 300 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.
9. **Pipette 150 µL** of TMB substrate into each well (the use of a multi-channel pipette is recommended).
10. **Incubate** the microplate inside a **37°C incubator** for **10-15 minutes**.
11. **Pipette 50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.
12. Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

CALCULATIONS

1. Calculate the mean optical density of each standard, control, and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a standard curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the standard curve.

QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The standard A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The standard with the highest concentration meets the % binding acceptable range as stated in the QC Certificate. % Binding = (OD of standard / OD of standard A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

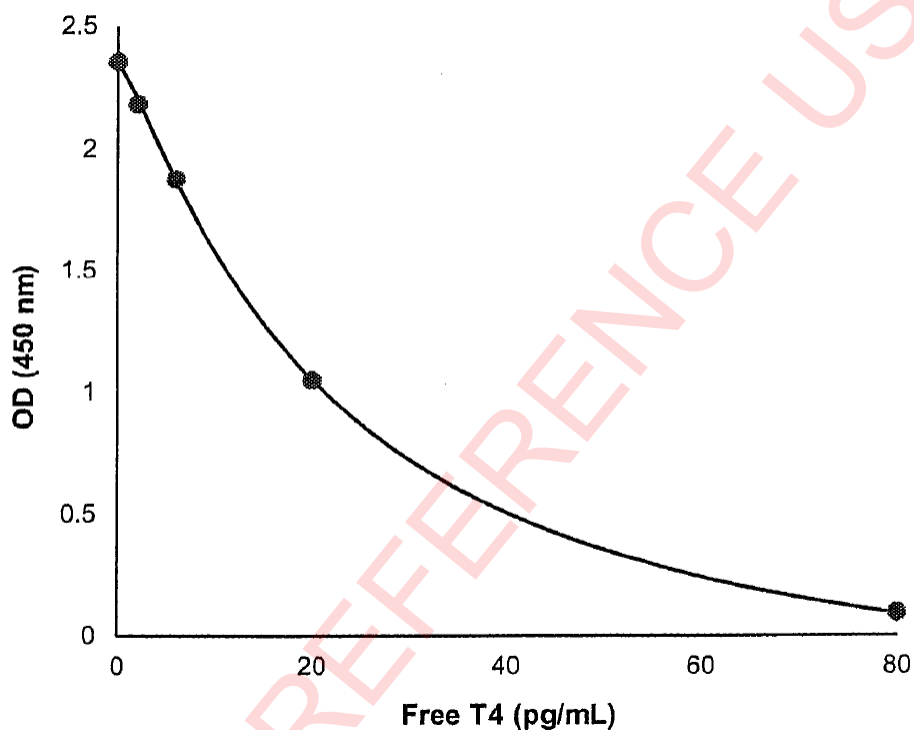
TYPICAL DATA

Sample data only. Do not use to calculate results.

Standard	Mean OD (450 nm)	% Binding	Value (Pg/mL)
A	2.354	100	0
B	2.180	93	2
C	1.875	80	6
D	1.046	44	20
E	0.091	4	80
Unknown	1.185	-	16.9

TYPICAL STANDARD CURVE

Sample curve only. Do not use to calculate results.



WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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