Package Insert

Free Thyroxine (Free T4) ELISA Assay kit

Catalog Number: T4F31-K01

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

v. 7.3 (20 FEB 24)

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INTENDED USE

The Eagle Biosciences Thyroxine (T4) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of thyroxine in human serum. The Eagle Biosciences Thyroxine (T4) ELISA Assay 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.

INTRODUCTION

Thyroxine (T4), the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. However, only the free (unbound) fraction of thyroxine is considered to be biologically active. The main carriers of thyroxine are thyroxine-binding globulin (TBG), pre-albumin and albumin. The measurement of free thyroxine (fT4) levels correlate better with the clinical status than total thyroxine levels. The free T4 assay is a one step competitive ELISA system that is rapid and easy to perform compared to equilibrium dialysis and ultrafiltration methods, which are cumbersome and time-consuming. This system employs a highly specific monoclonal antibody and a non-analog tracer that was proved experimentally to have no significant binding to TBG and albumin. In the euthyroid, normal population the free T4 concentration is 7-22 pg/mL. The level of free T4 is decreased in hypothyroidism while in thyrotoxic patients the level of free T4 is increased

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, controls and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microplate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the color formed is inversely proportional to the concentration of T4 in the sample. A set of standards is used to plot a standard curve from which the amount of T4 in patient samples and controls can be directly read.
The labeled T4 (conjugate) employe in this assay system has shown no binding properties towards thyroxine-binding globulin (TBG) and human serum (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 CAUTIONS AND WARNINGS

1. 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.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.

4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. 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.
10. The substrate solution (TMB) 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.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of T4 in human serum. The kit is not calibrated for the determination of T4 in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Samples reading higher than 100 pg/mL should be reported as such and should not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
5. The interpretation of free T4 results can be complicated by a variety of drugs, severe nonthyroidal illness and some rare conditions such as familial dysalbuminemic hyperthyroxinemia (FDH).
6. Some individuals may have antibodies to mouse protein that can possibly interfere with this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision pipettes to dispense 20, 50, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. A 37ºC incubator
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

MATERIALS PROVIDED

1. **Microplate** — Ready To Use

Contents:	One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage:	Refrigerate at 2–8°C
Stability:	12 months or as indicated on label.

2. **(HRP) Conjugate Concentrate** — Requires Preparation x51

Contents:	One bottle containing T4-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative
Volume:	0.3 mL/bottle

Storage: Refrigerate at 2–8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:51 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. **Calibrators** — Ready To Use

Contents: Five vials of calibrator 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 concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
A	0 pg/mL	0.5 mL
B	2 pg/mL	0.5 mL
C	6 pg/mL	0.5 mL
D	20 pg/mL	0.5 mL
E	80 pg/mL	0.5 mL

Storage: Refrigerate at 2–8°C.
Stability: Unopened: Stable until the expiration date on label
After Opening: Stabel for four weeks.

4. Controls — Ready to Use

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 T4. Refer to QC for target values and acceptable ranges.
Volume: 0.5 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: Unopened: Stable until the expiration date on label
After Opening: Stabel for four weeks.

5. **Wash Buffer Concentrate** — Requires Preparation x10

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: Refrigerate at 2–8°C
Stability: Unopened: Stable until the expiration date on label
After Opening: Stabel for four weeks.
Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **Assay Buffer** – Ready to Use

Contents:	One bottle containing a protein-based buffer with a non-mercury preservative.
Volume:	15 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	Unopened: Stable until the expiration date on label After Opening: Stabel for four weeks.

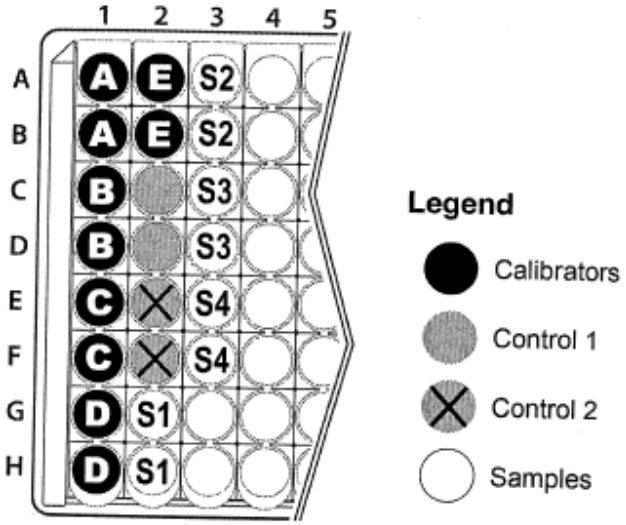
7. **TMB Substrate** — Ready To Use

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume:	16 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	Unopened: Stable until the expiration date on label After Opening: Stabel for four weeks.

8. **Stopping Solution** — Ready To Use

Contents:	One bottle containing 1M sulfuric acid.
Volume:	6 mL/bottle
Storage:	Refrigerate at 2–8°C
Stability:	Unopened: Stable until the expiration date on label After Opening: Stabel for four weeks.

RECOMMENDED ASSAY LAYOUT



ASSAY PROCEDURE

Specimen Pretreatment: None. All reagents must reach room temperature before use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the fT4-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. **Pipette 25 µL** of each calibrator, control, and specimen sample into correspondingly labelled wells in duplicate.
4. **Pipette 100 µL** of the HRP conjugate working solution into each well. (We recommend using a multichannel pipette).
5. Gently **shake** the plate for 10 seconds.
6. **Incubate** the plate at 37°C for 1 hour.
7. **Wash** the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.)
8. **Pipette 150 µL** of TMB substrate into each well at timed intervals.
9. **Incubate** the plate at 37°C for 10–15 minutes (or until calibrator A attains dark blue color for desired OD).
10. **Pipette 50 µL** of stopping solution into each well at the same timed intervals as in step 8.
11. **Read** the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution. * If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

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

QUALITY CONTROL

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

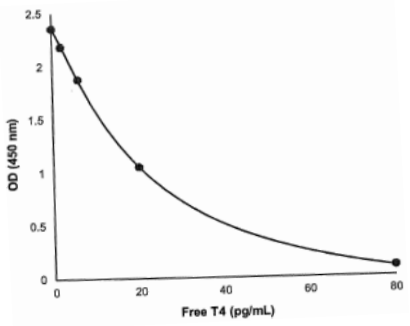
1. The calibrator A mean optical density meets the acceptable range as stated in the QC certificate.

2. The calibrator with the highest concentration meets the % binding acceptabl;e range as stated in the QC certificate. % Binding = (OD of calibrator/OD of calibrator 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 TABULATED DATA

Calibrator	Mean OD	% Binding	Value (ng/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 CALIBRATOR CURVE



WARRANTY INFORMATION

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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.