



**EAGLE**  
BIOSCIENCES

# THYROXINE (T4) ELISA

Catalog Number: T4T31-K01

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

*v. 2 (15 OCT 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](http://www.EagleBio.com) or at 866-411-8023.*

## ASSAY PRINCIPLE

The T4 ELISA is a competitive immunoassay. Competition occurs between T4 present in calibrators, 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 T4 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 calibrators is used to plot a calibrator curve from which the amount of T4 in specimen samples and controls can be directly read.

## PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:
  - a. Do not pipette by mouth.
  - b. Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
  - c. Wear protective clothing and disposable gloves.
  - d. Wash hands thoroughly after performing the test.
  - e. Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. 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.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. 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.
7. 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.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.
11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in 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 procedural techniques or pipetting, incomplete washing, or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.



14. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. The Assay Buffer is sensitive to light and should be stored the original dark bottle away from direct sunlight.
16. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
17. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
18. Samples values above the measuring range of the kit should be reported as >32% (ug/dL) and must not be diluted. Dilution will alter the existing equilibrium and may lead to false results.
19. Avoid microbial contamination of reagents.
20. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control
21. To prevent the contamination of reagents, do not pour reagents back into the original containers.
22. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
23. 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.
24. 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.
25. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
26. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
27. 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 shaker and/or speed is used, the user is responsible for validating the performance of the kit.
28. Do not reuse the microplate wells, they are for SINGLE USE only.
29. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
30. 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.

### **POTENTIAL BIOHAZARDOUS 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, STORAGE AND PRE-TREATMENT

### SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4-5 ml of venous blood into an appropriately labelled 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.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

### SPECIMEN PRE-TREATMENT

Specimen pre-treatment is not required.

### MATERIALS PROVIDED

1. Precision pipettes to dispense 20, 50, 150 and 300  $\mu$ L
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
  - Orbital shaker (3mm diameter) set to 600rpm
  - Reciprocating shaker (1.5" stroke length) set to 180 O/pm
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)
6. Automatic Plate Washer (recommended)

### REAGENTS PROVIDED

1. **Mouse Anti-T4 Antibody-Coated Break-Apart Well 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. **T4 Horseradish Peroxidase (HRP) Conjugate Concentrate — Requires Preparation X25**  
**Contents:** T4-HRP conjugate in a protein-based buffer with a non-mercury preservative.  
**Volume:** 1 mL/vial  
**Storage:** Refrigerate at 2–8°C  
**Stability:** 12 months or as indicated on label.  
**Preparation:** Dilute 1:25 in assay buffer before use (eg. 80  $\mu$ L of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 800  $\mu$ L of HRP in 20 mL of assay buffer. Discard any that is left over.



### 3. **T4 Calibrators — Ready To Use**

**Contents:** Five vials containing T4 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of T4.

\* Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator A	0 µg%	2.0 mL
Calibrator B	1 µg%	0.5 mL
Calibrator C	4 µg%	0.5 mL
Calibrator D	12 µg%	0.5 mL
Calibrator E	32 µg%	0.5 mL

**Storage:** Refrigerate at 2–8°C.

**Stability:** 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

### 4. **Controls — Ready To Use**

**Contents:** Two vials containing T4 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of T4. Refer to vial labels for the acceptable range.

**Volume:** 0.5 mL/vial

**Storage:** Refrigerate at 2–8°C

**Stability:** 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

### 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:** 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the 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:** 25 mL/bottle

**Storage:** Refrigerate at 2–8°C

**Stability:** 12 months or as indicated on label.

### 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/vial

**Storage:** Refrigerate at 2–8°C

**Stability:** 12 months or as indicated on label.



## 8. **Stopping Solution** – Ready to Use

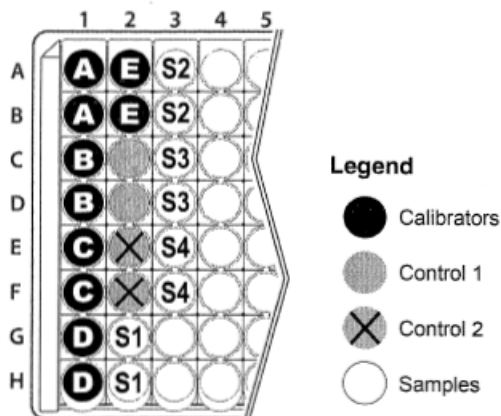
**Contents:** One bottle containing 1M sulfuric acid.

**Volume:** 6 mL/bottle

**Storage:** Refrigerate at 2–8°C

**Stability:** 12 months or as indicated on label.

## 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 T4-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 **20 µL** of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette **150 µL** of the conjugate working solution into each well. (We recommend using a multichannel pipette).
5. **Incubate** on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
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** on a plate shaker for 15–20 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
10. Pipette **50 µL** of stopping solution into each well at the same timed intervals as in step 7.
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.
4. If a sample reads more than 32% ng/ml and needs to be diluted and retested, then dilute with calibrator A not more than 1:8. The result obtained must be multiplied by the dilution factor.
5. Units:  $\mu\text{g}\%$  =  $\mu\text{g}/\text{dL}$ .

## QUALITY CONTROL

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

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

## TYPICAL DATA

### TYPICAL TABULATED DATA

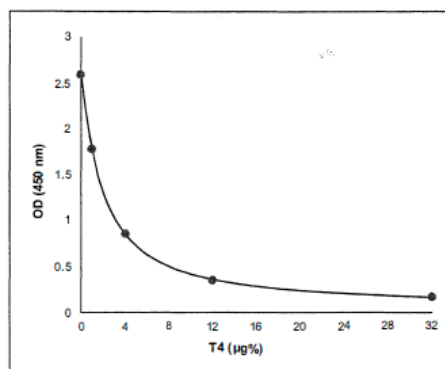
Sample data only. **Do not** use to calculate

Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	2.590	100	0
B	1.780	69	1
C	0.857	33	4
D	0.347	13	12
E	0.168	6	32
Unknown	0.401	-	10.4



## TYPICAL CALIBRATOR CURVE

Sample data only. **Do not** use to calculate



## 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](mailto:info@eaglebio.com) or at 866-411-8023.*