EAGLE BIOSCIENCES

# High Sensitive Anti-Tg IgG ELISA Assay Kit

Catalog Number:
ATG31-K01 (1 x 96 wells)
*For Research Use Only. Not for use in diagnostic procedures.*
*v. 1.0*

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## INTENDED USE

This Eagle Biosciences microplate-based ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human anti-Tg autoantibody (IgG) level in serum. The Eagle Biosciences anti-Tg autoantibody IgG ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## SUMMARY OF PHYSIOLOGY

It is a routine practice of measuring serum autoantibodies to thyroglobulin (Tg) and microsomal (TPO) for aid in detecting and monitoring autoimmune thyroid disease. Serum anti-TPO autoantibody and anti-Tg autoantibody are found to be well- correlating with histological changes in Harshimoto's thyroiditis. Clinically, positive anti-TPO autoantibody is detected in patients with chronic thyroiditis (70-90%), primary hypothyroidism (~60%), thyrotoxicosis (~50%) and thyroid tumors (~17%), however, anti-Tg autoantibody is mainly identified in patients with Harshimoto's thyroiditis and Graves' disease (40-70%).

Although ELISA technology has applied to detecting these autoantibodies, the high background in normal population would decrease the clinical diagnostic sensitivity and specificity. This high sensitive anti-Tg autoantibody ELISA kit was developed with proprietary technology that leads to a very low reaction background in normal population and thus would increase the clinical diagnostic sensitivity and specificity.

## ASSAY PRINCIPLE

This anti-Tg autoantibody IgG ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human anti-Tg IgG autoantibody level in test sample. Assay calibrators, controls and pre-diluted human serum samples containing anti-Tg IgG autoantibody are added to microtiter wells of microplate that was coated with high affinity streptavidin on its wall. The autoantibody reaction will not start until the addition of a biotinylated human Tg antigen. After the first incubation period, the unbound protein matrix is removed in the subsequent washing step. A horseradish peroxidase-conjugated rabbit anti-human IgG subclass specific antibody (tracer antibody) is added to each well.

After an incubation period an immunocomplex of "solid-phase bound biotin-Tg – human anti-Tg IgG – HRP-conjugated tracer antibody" is formed if there is human anti-Tg IgG autoantibody present in the test sample. The unbound tracer antibody is removed in the subsequent washing step. HRP-conjugated tracer antibody bound to the well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the tracer antibody bound to the human IgG on the wall of the microtiter well is directly proportional to the amount of human anti-Tg IgG autoantibody level in the sample. Plotting the absorbance versus the respective human anti-Tg IgG autoantibody concentration for each calibrator on point-to-point or 4-parameter fit generates a calibrator curve. The concentration of human anti-Tg IgG autoantibody in test samples is determined directly from this calibrator curve.

**REAGENTS: Preparation and Storage**
This anti-Tg autoantibody IgG ELISA Assay Kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date on the anti-Tg autoantibody IgG ELISA Assay Kit box.

**Prior to use allow all reagents to come to room temperature.** Reagents from different kit lot numbers should not be combined or interchanged.

1. **Streptavidin Coated Microplate**

One microplate with 12 x eight strips (96 wells total) coated with streptavidin. The plate is framed and sealed in a foil Ziploc bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **Biotinylated Tg**

One vial containing 10 mL of ready-to-use biotinylated human Tg solution in a stabilized matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **Anti-hIgG Tracer Antibody**

One vial containing 0.6 mL concentrated horseradish peroxidase (HRP)-conjugated anti-human IgG tracer antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **Tracer Antibody Diluent**

One vial containing 12 mL ready-to-use buffer. It should be only used for antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **ELISA Wash Concentrate**

One bottle contains 30 mL of 30-fold concentrate. Before use the contents must be diluted with 870 mL of distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted wash solution should be stored at room temperature and is stable until the expiration date on the kit box.

6. **ELISA HRP Substrate**

One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. **ELISA Stop Solution**

One bottle contains 12 mL of 0.5 M sulfuric acid. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

8. **Anti-Tg hIgG Calibrators**

Five vials each contain anti-Tg IgG type autoantibody in a liquid bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration for each calibrator.** After the first use, the calibrators should be stored at -20°C or below for long-term storage.

**9. Anti-Tg hIgG Controls**

Two vials each contain anti-Tg IgG type autoantibody in a liquid bovine serum albumin-based matrix with a non-azide preservative. **Refer to vials for exact concentration range for each control**. After the first use, the calibrators should be stored at -20°C or below for long-term storage.

**10. Sample Diluent**

Two bottles each contain 30 mL phosphate buffer with protein stabilizers and preservative. The reagent is ready to use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

## SAFETY PRECAUTIONS

The reagents of the anti-Tg autoantibody IgG ELISA Assay Kit must be used in research or clinical laboratory and are for professional use only. Reagents of bovine serum albumin were derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 50 µL, 100 µL, and 1000 µL, etc.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm or 13 x 100 glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Deionized or distilled water.
8. Plastic microtiter well cover or polyethylene film.
9. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
10. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

## SPECIMEN COLLECTION

Only 10 µL of human serum is required for anti-Tg autoantibody measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected and must be allowed to clot for minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within three hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 – 8°C up to 48 hours and at –20°C or below for long-term storage until measurement.

## ASSAY PROCEDURE

### 1. Sample Preparation

Sample needs to be diluted 1:101 with Sample Diluent before being measured.

(1) Label a test tube (12x75 mm).
(2) Add 1 mL of the diluent into tube. Pipet 10 µL of serum sample to the tube and mix well.

### 2. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to working solution prior to use. Please see REAGENTS section for details.

### 3. Assay Procedure

1. Place a sufficient number of streptavidin-coated microwell strips in a holder to run anti-Tg hIgG calibrators, controls and pre-diluted unknown samples in duplicate.
2. Test Configuration

| ROW | STRIP 1 | STRIP 2 | STRIP 3 |
|---|---|---|---|
| **A** | STD 1 | STD 5 | SAMPLE 2 |
| **B** | STD 1 | STD 5 | SAMPLE 2 |
| **C** | STD 2 | C 1 | SAMPLE 3 |
| **D** | STD 2 | C 1 | SAMPLE 3 |
| **E** | STD 3 | C 2 | SAMPLE 4 |
| **F** | STD 3 | C 2 | SAMPLE 4 |
| **G** | STD 4 | SAMPLE 1 | SAMPLE 5 |
| **H** | STD 4 | SAMPLE 1 | SAMPLE 5 |

3. Add **25 µL** of calibrators, controls and diluted patient serum samples into the designated microwell.
4. Add **100 µL** of biotinylated Tg solution into each well.
5. Cover the plate with one plate sealer.
6. Incubate plate at room temperature for **1 hour**.
7. Prepare Anti-hIgG Tracer Antibody Working Solution by **1:21 fold** dilution of the tracer antibody with the Tracer Antibody Diluent. For each strip, it is required to mix 1 mL of Tracer Antibody Diluent with 50 µL of the Tracer Antibody in a clean test tube.
8. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
9. Add **100 µL** of above diluted tracer antibody working solution to each of the wells.
10. Cover the plate with one plate sealer and also with aluminum foil to avoid exposure to light.
11. Incubate plate at room temperature for **30 minutes**.
12. Remove the plate sealer. Aspirate the contents of each well. Wash each well 5 times by dispensing 350 µL to 400 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
13. Add **100 µL** of ELISA HRP Substrate into each of the wells.

14. Cover the plate with a new plate sealer and also with aluminum foil to avoid exposure to light.
15. Incubate plate at room temperature for 20 minutes.
16. Remove the aluminum foil and plate sealer. Add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
17. Read the absorbance at 450 nm within 10 minutes in a microplate reader.

**PROCEDURAL NOTES**

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the anti-Tg autoantibody IgG ELISA test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
7. All reagents of the anti-Tg autoantibody IgG ELISA Assay Kit should be mixed gently and thoroughly prior to use. Avoid foaming.

**INTERPRETATION OF RESULTS**

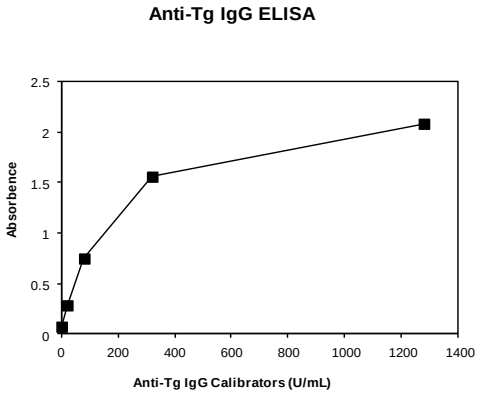
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the calibrator 1 (0 U/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The calibrator curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The anti-Tg hIgG concentrations for the controls and samples are read directly from the calibrator curve using their respective corrected absorbance.

**EXAMPLE DATA AND CALIBRATOR CURVE**

A typical absorbance data and the resulting calibrator curve from human anti-Tg AAB-IgG EIA are represented. **This curve should not be used in lieu of calibrator curve run with each assay.**

| OD 450 nm Absorbance | | | | |
|---|---|---|---|---|
| **Well / I.D** | **Readings** | **Average** | **Corrected** | **Results (U/mL)** |
| 0 U/mL | 0.074<br>0.071 | 0.072 | 0 | |
| 20 U/mL | 0.28<br>0.289 | 0.284 | 0.212 | |
| 80 U/mL | 0.751<br>0.745 | 0.748 | 0.676 | |
| 320 U/mL | 1.538<br>1.582 | 1.56 | 1.488 | |
| 1280 U/mL | 2.1<br>2.071 | 2.085 | 2.013 | |
| Control 1 | 0.206<br>0.197 | 0.201 | 0.129 | 12.15 U/mL |
| Control 2 | 1.058<br>1.037 | 1.047 | 0.975 | 168.48 U/mL |



## EXPECTED VALUES

Serum from 128 normal adults, as well as 60 patients with thyroid diseases were measured with this EIA. The following is a guide to interpretation of results. Because the prevalence of human anti-Tg IgG antibodies may vary depending on a number of factors such as age, gender, geographical location, race, type of test used and clinical history of individual patients, it is strongly recommended that each laboratory should establish its own "normal" range based on populations encountered.

| Unit Value | Interpretation |
|---|---|
| < 30 U/mL | Negative |
| 30 – 50 U/mL | Borderline |
| > 50 U/mL | Positive |

## LIMITATION OF THE PROCEDURE

(1) The results obtained with the anti-Tg IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in itself.
(2) Since there is no Gold Standard concentration available for anti-Tg IgG measurement, the values of assay calibrators were established and calibrated in arbitrary units (U/mL).
(3) For unknown sample value read directly from the assay that is greater than 320 U/mL, it is recommended to measure a further diluted sample for more accurate measurement.
(4) Bacterial or fungal contamination of serum specimens or reagents, or cross-contamination between reagents may cause erroneous results.
(5) Water deionized with polyester resins may inactive the horseradish peroxidase enzyme.

## QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known anti-Tg IgG levels. We recommend that all assays include the laboratory's own controls in addition to those provided with this anti-Tg autoantibody IgG ELISA Assay Kit.

## PERFORMANCE CHARACTERISTICS

### Sensitivity

The sensitivity of this anti-Tg IgG EIA as determined by the 95% confidence limit on 20 duplicate determination of zero calibrator is about 1 U/mL.

### Precision

The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations.

| Mean Anti-Tg IgG Value (U/mL) | CV (%) |
|---|---|
| 12.5 | 5.7 |
| 165.8 | 3.8 |

The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays.

| Mean Anti-Tg IgG Value (U/mL) | CV (%) |
|---|---|
| 11.6 | 7.2 |
| 159.2 | 5.4 |

**Specificity**
This assay specifically detects human anti-Tg antibody and no cross-reactivity to other autoantibodies has been observed.

**Linearity**
Two positive samples were diluted with assay buffer and assayed. The results in the value of U/mL are as follows:

| # | DILUTION | OBSERVED VALUE | EXPECTED VALUE | RECOVERY % |
|---|---|---|---|---|
| 1 | Original | 156.2 | - | - |
| | 1:2 | 82.4 | 78.1 | 106 |
| | 1:4 | 41.2 | 39.1 | 105 |
| | 1:8 | 19.2 | 19.5 | 98 |
| 2 | Original | 62.8 | - | - |
| | 1:2 | 30.2 | 31.4 | 96 |
| | 1:4 | 14.6 | 15.7 | 93 |
| | 1:8 | 7.1 | 7.9 | 90 |

**REFERENCES**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*Short Assay Procedure*

1. Add **25 µL** of assay calibrators, controls and 1:101 diluted patient samples into each well
2. Add **100 µL** of biotinylated Tg solution into each well
3. Incubate the plate at RT for **1 hour**
4. Wash plate 3 times with wash buffer
5. Add **1:21** fold diluted anti-Tg hIgG tracer antibody into each well
6. Incubate the plate at RT for **30 minutes**
7. Wash plate 3 times with wash buffer
8. Add **100 µL** of TMB substrate into each well
9. Incubate the plate at RT for 20 minutes
10. Add **100 µL** of stop solution into each well
11. Read the plate at OD 450 nm

**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.