EPITOPE DIAGNOSTICS, INC.

EDI™ High Sensitive Anti-TPO IgG ELISA Kit

Enzyme Linked Immunosorbent Assay (ELISA) for the measurement of human anti-TPO autoantibody (IgG type) level in serum

REF KT-833 CE IVD 96

INTENDED USE

This microplate based ELISA (enzyme-linked immunosorbent assay) kit is intended for the quantitative determination of human anti-TPO autoantibody (IgG) level in serum. The presence of this autoantibody together with clinical findings and other laboratory tests is a useful tool in the aid of diagnosis of autoimmune thyroid disease. This kit is for in vitro diagnostic use only.

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 EDI™ anti-TPO 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 ELISA is designed, developed, and produced for the quantitative measurement of human anti-TPO IgG level in test sample. The assay utilizes the streptavidin coated microplate based enzyme immunoassay technique.

Assay calibrators, controls and pre-diluted human serum samples containing anti-TPO IgG 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 TPO 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 -TPO – human anti-TPO IgG – HRP-conjugated tracer antibody" is formed if there is human anti-TPO 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-TPO IgG autoantibody level in the sample. Plotting the absorbance versus the respective human anti-TPO IgG autoantibody concentration for each calibrator on point-to-point or 4-parameter fit generates a calibrator curve. The concentration of human anti-TPO IgG autoantibody in test samples is determined directly from this calibration curve.

REAGENTS: PREPARATION AND STORAGE

This test 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.

1. TPO Coated Microplate (31312)

Microplate coated with streptavidin.

Qty: 1 x 96 well microplate
Storage: 2 – 8°C
Preparation: Ready to Use.

2. Anti-hIgG Tracer Antibody (30051)

Concentrated horseradish peroxidase (HRP) conjugated anti-human IgG tracer antibody in a stabilized protein matrix.

Qty: 1 x 0.6 mL
Storage: 2 – 8°C
Preparation: 21X Concentrate. The contents must be diluted with tracer antibody diluent (30052) and mixed well before use.

3. Tracer Antibody Diluent (30052)

Concentrated horseradish peroxidase (HRP) conjugated anti-human IgG tracer antibody in a stabilized protein matrix.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

4. ELISA Wash Concentrate (10010)

Surfactant in a phosphate buffered saline with non-azide preservative.

Qty: 1 x 30 mL
Storage: 2 – 8°C
Preparation: 30X Concentrate. The contents must be diluted with 870 mL distilled water and mixed well before use.

5. ELISA HRP Substrate (10020)

Tetramethylbenzidine (TMB) with stabilized hydrogen peroxide.

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

6. ELISA Stop Solution (10030)

0.5 M sulfuric acid

Qty: 1 x 12 mL
Storage: 2 – 8°C
Preparation: Ready to Use

7. Anti-TPO hIgG Calibrators Levels 1- 5 (30104 - 30108)

Anti-TPO IgG type autoantibody in a liquid bovine serum albumin-based matrix with a non azide preservative. Refer to vials for exact concentration range.

Qty: 5 x Vials
Storage: 2 – 8°C , <-20°C Long term storage
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use.

8. Anti-TPO hIgG Controls (30109, 30110)

Anti-TPO IgG type autoantibody in a liquid bovine serum albumin-based matrix with a non azide preservative. Refer to vials for exact concentration range.

Qty: 2 x Vials
Storage: 2 – 8°C , <-20°C Long term storage
Do not exceed 3 freeze-thaw cycles.
Preparation: Ready to Use.

9. Patient Sample Diluent (30905)

Phosphate buffer with protein stabilizers and preservative.

Qty: 2 x 30 mL
Storage: 2 – 8°C
Preparation: Ready to Use.

SAFETY PRECAUTIONS

The reagents are for in vitro diagnostic use only. Source material which contains reagents of bovine serum albumin was 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 were potentially infectious. Avoid contact with reagents containing hydrogen peroxide, or sulfuric acid. 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 & STORAGE

Only 10 µL of human serum is required for anti-TPO autoantibody measurement in duplicate. Samples should not be taken from patients taking biotin-containing multivitamins or dietary supplements at least 48 hours 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. 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 (10010) must be diluted to working solution prior use. Please see REAGENTS section for details.

2. Sample Preparation

1. Patient sample needs to be diluted 1:101 with Patient Sample Diluent (30049) before being measured.
2. Label a test tube (12x75 mm).
3. Add 1 mL of the diluent into each tube. Pipette 10 µL of patient serum sample to the tube and mix well.

3. Assay Procedure

1. Place a sufficient number of microwell strips (10400) in a holder to run calibrators (30104-30108), controls (30109,30110), and samples in duplicate.
2. Test Configuration

Row	Strip 1	Strip 2	Strip 3
A	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
B	Calibrator Level 1	Calibrator Level 5	SAMPLE 2
C	Calibrator Level 2	Control 1	SAMPLE 3
D	Calibrator Level 2	Control 1	SAMPLE 3
E	Calibrator Level 3	Control 2	SAMPLE 4
F	Calibrator Level 3	Control 2	SAMPLE 4
G	Calibrator Level 4	SAMPLE 1	SAMPLE 5
H	Calibrator Level 4	SAMPLE 1	SAMPLE 5

3. Add **50 µL** of calibrators (30104-30108), controls (30109,30110), and diluted samples into the designated microwells.
4. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **40 minutes.**
5. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and completely aspirate the contents. Alternatively, an automated microplate washer can be used.
6. Prepare the antibody working solution by 1:21 fold dilution of the tracer antibody (30051) with the diluent (30052). For each strip, it is required to mix **1 mL** of the diluent (30052) with **50 µL** of the tracer antibody (30151) in a clean test tube. *Note: This antibody working solution should be freshly prepared.*
7. Add **100 µL** of antibody working solution to each microwell. Mix by gently tapping the plate.
8. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **30 minutes.**
9. Remove the plate sealer. Aspirate the contents of each well. Wash each well **5 times** by dispensing **350 µL** of diluted wash solution (10010) into each well, and completely aspirate the contents. Alternatively, an automated microplate washer can be used.

10. Add **100 µL** of substrate (10020) into each microwell. Mix by gently tapping the plate.
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
12. Remove the aluminum foil and plate sealer and add **100 µL** of Stop Solution (10030) into each of the wells. Mix by gently tapping the plate.
13. Read the absorbance at **450/620 nm** within **10 minutes** with 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 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 should be mixed gently and thoroughly prior to use. Avoid foaming.

INTERPRETION 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-TPO hIgG concentrations for the controls and samples are read directly from the calibrator curve using their respective corrected absorbance. If log-log graph paper or computer assisted data reduction program utilizing logarithmic transformation are used, sample having corrected absorbance between the level 2 calibrator and the next highest calibrator should be calculated by the formula:

$$\text{Value of unknown} = \frac{\text{Corrected absorbance (unknown)}}{\text{Corrected Absorbance (2nd level)}} \times \text{Value of the 2nd level}$$

LIMITATIONS OF THE PROCEDURE

1. The results obtained with the anti-TPO 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-TPO IgG measurement, the values of assay calibrators were established and calibrated in arbitrary U/mLs (U/mL).
3. For unknown sample value read directly from the assay that is greater than 200 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-TPO IgG levels. We recommend that all assays include the laboratory's own controls in addition to those provided with this kit.

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-TPO 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.

U/mL Value	Interpretation
< 9.0 U/mL	Negative
≥ 9.0 U/mL	Positive

EXAMPLE DATA

A typical absorbance data and the resulting calibrator curve from are represented.

Note: This curve should not be used in lieu of calibrator curve run with each assay.

Well ID	Reading Absorbance (450 nm)			Concentration (U/mL)
	Readings	Average	Corrected	
Calibrator Level 1: 0 U/mL	0.077	0.086	0.000	
	0.095			
Calibrator Level 2: 15 U/mL	0.315	0.329	0.243	
	.0342			
Calibrator Level 3: 60 U/mL	0.807	0.803	0.717	
	0.799			
Calibrator Level 4: 240 U/mL	1.768	1.821	1.735	
	1.874			
Calibrator Level 5: 960 U/mL	2.735	2.745	2.659	
	2.756			
Control 1	0.198	0.205	0.119	7.36
	0.212			
Control 2	1.052	1.055	0.969	104.46
	1.057			

PERFORMANCE CHARACTERISTICS

Sensitivity

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

Specificity
This ELISA is specific for the measurement of human anti-TPO IgG. No cross-reactivity to other autoantibodies has been observed.

Reproducibility and Precision
The intra-assay precision is validated by measuring two samples in a single assay with 20 replicate determinations. The inter-assay precision is validated by measuring two samples in duplicate in 12 individual assays. The results are as follows:

	Intra-Assay		Inter-Assay	
Sample	**1**	**2**	**1**	**2**
Mean (U/mL)	7.5	102.6	7.3	105.9
CV (%)	6.4	4.9	7.2	5.6

Linearity
Two human serum samples were diluted with assay buffer and assayed. The results are as follows:

Samples	Observed (U/mL)	Expected (U/mL)	Recovery (%)
Sample A	68.2	-	-
50%	32.6	34.1	96
25%	16.2	17.1	95
12.5%	7.9	8.5	93
Sample B	26.8	-	-
50%	14.2	13.4	106
25%	6.4	6.7	96
12.5%	3.2	3.4	94

WARRANTY
This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.

This product is developed and manufactured by

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Please visit our website at www.epitopediagnostics.com to learn more about our products and services.

EC	REP

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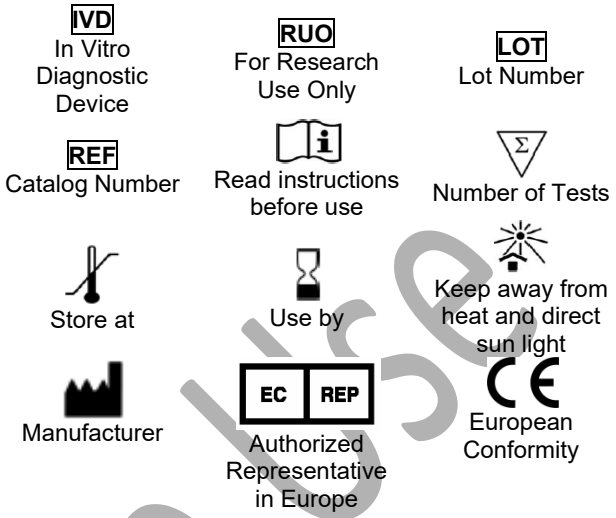
GLOSSARY OF SYMBOLS (EN 980/ISO 15223)



SHORT ASSAY PROCEDURE
1. Add **25 µL** of calibrators, controls, and diluted samples into the designated microwells.
2. Add **25 µL** of biotinylated TPO into the designated microwells. Mix by gently tapping the plate.
3. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **60 minutes**.
4. Wash each well five times
5. Add **100 µL** of antibody working solution to each microwell. Mix by gently tapping the plate.
6. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **30 minutes**.
7. Wash each well five times
8. Add **100 µL** of substrate into each well.
9. Mix, cover, and incubate at **room temperature (20-25 ºC)** for **20 minutes**.
10. Wash each well five times
11. Cover the plate with one plate sealer and aluminum foil. Incubate at **room temperature (20-25 ºC)** for **20 minutes.**
12. Add **100 µL** of the stop solution to each well.
13. Read the absorbance at **450 nm**.