

ELISA Kit...The EiAsy™ Way
ANTI-THYROGLOBULIN (ANTI-TG)
AUTOANTIBODIES

REF CAN-TGAb-4000 Version: 3.0

Effective: March 28, 2005



INTENDED USE

For the quantitative determination of anti-thyroglobulin (anti-TG) autoantibodies (autoAbs) by enzyme immunoassay in human serum.

For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows a two-step assay. Purified human thyroglobulin (TG) antigen from the thyroid gland is immobilized onto the microwell plate. In the first incubation step, the autoantibodies to TG (present in standards, control and serum samples) bind specifically to the immobilized TG. The washing and decanting procedures remove unbound materials. In the second incubation, bound autoAbs are detected using an anti-human-IgG-peroxidase conjugate. After the second 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 proportional to the amount of anti-TG autoAbs. A set of standards is used to plot a standard curve from which the amount of anti-TG autoAbs in serum samples and controls can be directly read.

CLINICAL APPLICATIONS

Thyroglobulin (TG) is a major component of the thyroid follicular colloid. It is a glycoprotein with a molecular weight of 660 kDa, and is the precursor for the biosynthesis of the two major thyroid hormones, triiodothyronine and thyroxine (T3 and T4 respectively).

Disorders of the thyroid gland are frequently caused by an autoimmune reaction. Increased levels of anti-TG autoantibodies are present in 30 percent of patients with Graves' disease and 85 percent of patients with Hashimoto's thyroiditis. Autoantibodies to thyroid peroxidase (TPO) occur more frequently than anti-TG in these diseases. Lower levels of anti-TG are also found to exist in approximately 10 percent of healthy individuals.

The measurement of anti-TG helps to confirm the diagnosis of autoimmune diseases in the thyroid gland. In addition, it can also be useful in the measurement of thyroglobulin, due to the fact that anti-TG can interfere with the immunoassay for TG.

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.

- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
- 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.
- A calibrator curve must be established for every run.
- The control should be included in every run and fall within established confidence limits.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
- When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) 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.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- 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.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

- All the reagents within the kit are calibrated for the direct determination of anti-TG autoAbs in human serum. The kit is not calibrated for the determination of anti-TG autoAbs in saliva, plasma or other specimens of human or animal origin.
- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only Calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- Anti-TG may be found in approximately 10% of the normal population at a low level.
- The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS
POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and control has been tested and found to be non-reactive 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. However no test method 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

Dilute patient serum samples 1:100 in Calibrator A before use. In order to conserve reagent, a serial dilution is recommended. Example: To 180 µl of Calibrator add 20 µl of serum sample (1:10). To 30 µl of this 1:10 diluted sample, add 270 µl of Calibrator A.

*Do not dilute the standards and control, they are ready for use.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

- Precision pipettes to dispense 50, 100 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 12).

REAGENTS PROVIDED

1. Purified Human Thyroglobulin Coated Microwell Plate-Break Apart Wells - Ready To Use.

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

2. Rabbit Anti-Human IgG-Horseradish Peroxidase (HRP) Conjugate Concentrate – [X50]

Contents: Anti-human IgG-HRP conjugate in a protein-based buffer with a non-mercury preservative.
 Volume: 300 µl/vial
 Storage: Refrigerate at 2-8°C
 Stability: 12 months or as indicated on label.
 Preparation: Dilute 1:50 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 12ml of assay buffer. Discard any that is left over.

3. Human Anti-TG AutoAbs Calibrators - Ready To Use.

Contents: Five vials containing human anti-TG autoAbs in a buffer with a non-mercury preservative. Calibrated against 1st International Reference Standards MRC65/093.

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

Calibrator	Concentration	Volume/Vial
Calibrator A	0 IU/ml	50 ml
Calibrator B	5 IU/ml	1.0 ml
Calibrator C	40 IU/ml	1.0 ml
Calibrator D	300 IU/ml	1.0 ml
Calibrator E	2000 IU/ml	1.0 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. Control - Ready To Use.

Contents: One vial containing human anti-TG autoAbs in a buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of anti-TG autoAbs. Refer to vial label for expected value and acceptable range.

Volume: 1.0 ml/vial

Storage: Refrigerate at 2-8°C

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

5. Wash Buffer Concentrate - [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: Four vials containing a protein-based buffer with a non-mercury preservative.

Volume: 15 ml/kit

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/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

8. Stopping Solution - Ready To Use.

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment:

Dilute 1:100 With Calibrator A Before Use.

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 human anti-IgG-HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 100 µl of each calibrator, control and diluted specimen sample into correspondingly labelled wells in duplicate.
4. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
5. 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).
6. Pipette 100 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
7. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
8. Wash wells 3 times as in step 5.
9. Pipette 100 µl of TMB substrate into each well at timed intervals.
10. Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator E attains dark blue colour for desired OD).
11. Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 9.
12. Read the plate on a microwell plate reader at 450nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

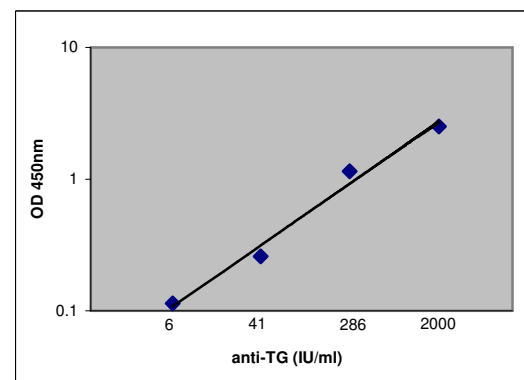
1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, control and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 2000 IU/ml (or the highest calibrator) then dilute it with Calibrator A at a dilution of no more than 1:8 (from original 1:100 dilution). The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (IU/ml)
A	0.088	0.091	0.090	0
B	0.111	0.117	0.114	6
C	0.217	0.255	0.259	41
D	1.129	1.169	1.149	286
E	2.568	2.446	2.507	2000
Unknown	0.116	0.131	0.124	8.1

TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The detection limit of an assay is commonly defined as the apparent concentration of 2 standard deviations above the mean of 10 replicates of zero standard. Therefore the sensitivity of the dbc anti-TG Autoantibodies ELISA kit is **3 IU/ml**.

SPECIFICITY (CROSS REACTIVITY)

The specificity of this assay is dependent upon the purity of the antigen coated onto the microtiter plate. The thyroglobulin used in this assay is over 98% pure, as determined by SDS-PAGE.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in IU/ml) are tabulated below:

Sample	Mean	SD	CV%
1	12.45	1.378	11.07
2	47.78	3.804	7.96
3	78.09	4.951	6.34

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in IU/ml) are tabulated below:

Sample	Mean	SD	CV%
1	13.78	1.669	12.11
2	54.67	5.396	9.87
3	89.23	8.611	9.65

RECOVERY

Spiked samples were prepared by adding defined amounts of anti-TG antibodies to three patient serum samples. The results (in IU/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	11.689	-	-
+50(10:2)	19.782	18.3	109
+400(10:2)	64.685	75.0	87
+2000(10:2)	268.459	343.0	80
2 Unspiked	8.089	-	-
+50(10:2)	13.642	15	93
+400(10:2)	61.853	73	85
+2000(10:2)	319.670	340	70
3 Unspiked	13.17	-	-
+50(10:2)	17.394	17.394	91
+400(10:2)	59.200	78	81
+2000(10:2)	327.093	344	95

LINEARITY

Three patient serum samples were diluted with assay buffer. The results (in IU/ml) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	233.333	-	-
1:2	124.026	117	106
1:4	59.244	58	102
1:8	23.632	29	83
2	113.945	-	-
1:2	67.594	61	111
1:4	38.646	31	125
1:8	18.480	16	116
3	112.016	-	-
1:2	64.347	56	114
1:4	34.006	28	121
1:8	15.070	14	107

EFFECT OF BILIRUBIN

Presence of bilirubin at concentrations up to 150 mg/ml had no significant effect on assay results.

EFFECT OF HEMOLYSIS

Presence of hemoglobin at concentrations up to 200 mg/dl had no significant effect on assay results.

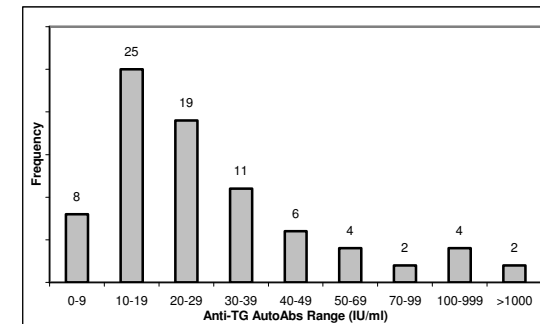
EFFECT OF LIPEMIA

Presence of triglycerides at concentrations up to 10 mg/ml had no significant effect on assay results.

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values. A study using the dbc anti-TG Autoantibodies ELISA kit on 81 serum samples from apparently health individuals showed a 90th percentile of 62 IU/ml, suggesting a normal range of: **0-62 IU/ml**.

The following graph shows the distribution of 81 subjects for various ranges of anti-TG autoAbs concentrations (IU/ml).



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