References: Czarnocka B et al. (1985) FEBS Lett. 190, 147-152; Libert F ET AL. (1987) EMBO J. 6, 4193 – 4196.

ELISA kits available from ADI (see details at the web site)

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#1190 Human Serum Albumin #1200 Human Albumin (Urinary)
#1750 Human IgG (total) #1760 Human IgM
#1800 Human IgE #1810 Human Ferritin
#1210 Human Transferrin (Tf) #0020 Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0030 Human Insulin #0040 Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #0410 HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) #1110 Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) #1700 Human T3 (total)

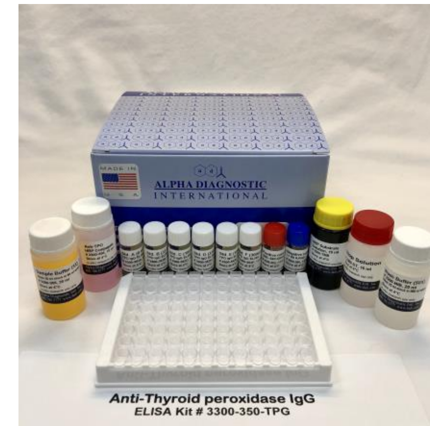
#1850 Human Cortisol #1860 Human Progesterone
#1865 Human Pregnolone #1875 Human Aldosterone
#1880 Human Testosterone #1885 Human free Testosterone
#1910 Human Androstenedione #1920 Human Estradiol
#1925 Human Estrone #1940 Dihydrotestosterone (DHT)

Instruction Manual No. M-3300-350-TPG

Human Anti-thyroid peroxidase (TPO) IgG ELISA KIT

Cat # 3300-350-TPG, 96 Tests

For Quantitative determination of IgG autoantibodies against TPO in Human Serum or plasma



For In Vitro Research Use Only (RUO)

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

TPO IgG ELISA Kit Cat # 3300-350-TPG

Kit Components	96 tests
TPO **coated microplate** (96 wells), #3300-351P	1 plate
Anti-TPO **Standards (A-F),** #3300-352A-F (0, 33, 100, 330, 1000 & 3000 IU/ml), 1.5 ml/vial	6 Vials
Anti- TPO **Positive control**, 1.5 ml, #3300-353	1 Vial
Anti- TPO **Negative control**, 1.5 ml, #3300-354	1 Vial
Sample Buffer (5X), 20 ml, #3300-355	1 Bottle
Wash buffer concn. (50X) , 20 ml, #3300-WB	1 Bottle
Anti-TPO HRP Conjugate, 15 ml, #3300-356	1 Bottle
HRP substrate Solution, 15 ml, # 3300-TMB	1 Bottle
Stop solution, 15 ml, #3300-ST	1 Bottle
Instruction Manual, M-3300-350-TPG	1

Intended Use

Human Anti-thyroid peroxidase (TPO) IgG ELISA kit is used for the quantitative measurement of IgG class autoantibodies against TPO in human serum or plasma.. **For in vitro research use only (RUO).**

Introduction

Thyroid disorders are the most prevalent of all autoimmune diseases. Thyroid autoimmune diseases are associated with the occurrence of differentiated autoantibodies and are thought to be related to a genetical pre-disposition. These autoantibodies are directed against membrane- located and/or extracellular antigens of the thyroid cells.

Human Thyroglobulin (hTG), a water soluble glycoprotein with a molecular weight of approx. 660.000 Dalton, is the principal constituent of the thyroidal colloid sharing about 75 % of its mass. Synthesis of the thyroid hormones T3 and T4 is based on the oxidative iodination of tyrosine residues of the thyroglobulin molecule. Within the cell thyroglobulin is transported by the microsomes. Together with the secretion of T3 and T4 also small amounts of hTG are liberated into circulation.

The microsomal antigen of the thyroid is an integral membrane protein of the microsomes. It has been characterized as the enzyme Thyroid peroxidase or thyroperoxidase (TPO) with a molecular weight of nearly 110.000 Dalton. TPO is an enzyme expressed mainly in the thyroid that liberates iodine for addition onto tyrosine residues on thyroglobulin for the production of thyroxin (T4) or triiodothyronine (T3), thyroid hormones. In humans, thyroperoxidase is encoded by the TPO gene.

TPO is stimulated by TSH, which up regulates gene expression. Thyroid peroxidase is a frequent epitope of autoantibodies in autoimmune thyroid disease. Such antibodies are called anti-thyroid peroxidase antibodies (anti-TPO antibodies), and are most commonly associated with Hashimoto's thyroiditis.

For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
3. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, ADI shall have no liability.
4. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

PERFORMANCE CHARACTERISTICS

Measuring range
0 - 3000 IU/ml

Interpretation of results

Negative:	<50 IU/ml
Borderline:	50-75 IU/ml
Positive:	>75 IU/ml

Limit of detection
5 IU/ml

Reproducibility

Intra-Assay

Sample	Mean (IU/ml)	CV %
1	337.0	1.6
2	835.0	2.9
3	2287.0	1.5

Inter-Assay

Sample	Mean (IU/ml)	CV %
1	324.0	3.1
2	761.0	3.5
3	2173.0	9.7

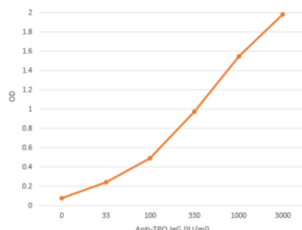
Interfering substances

No interference has been observed with hemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A450nm
A1, A2	Std. A (0 IU/ml)	0.077
B1, B2	Std. B (33 IU/ml)	0.243
C1, C2	Std. C(100 IU/ml)	0.494
D1, D2	Std. D (330 IU/ml)	0.976
E1, E2	Std. E (1000 IU/ml)	1.543
F1, F2	Std. F (3000 IU/ml)	1.980
G1, G2	Sample 1	0.85

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Calculation of results:

A dose response curve is used to ascertain the concentration of Anti-TPO IgG in unknown specimens.

1. Record the A450 obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the A450 for mean duplicate calibrator versus the Anti-TPO IgG concentration in IU/ml on linear graph paper. Connect the points with a best-fit curve.
3. To determine the concentration of Anti-TPO IgG for an unknown, locate the average A450 of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.85) intersects the dose response curve at 287.39 IU/ml Anti-TPO IgG concentration (See Figure 1).

Interpretation

1. *Measurements and interpretation of results must be performed by a skilled individual or trained professional.*
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
   The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and all other clinical findings.

PRINCIPLE OF THE TEST

Anti-TPO IgG ELISA kit is based on an indirect enzyme linked immune reaction where specific antibodies in the patient sample bind to the highly purified TPO antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of diluted sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (10-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate shaker (orbital shaker), plate washer (recommended) and ELISA plates Reader.

SPECIMEN COLLECTION AND STORAGE

The specimens shall be blood, serum in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) with no anti-coagulants. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8oC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.2 ml (200µl) of the specimen is required.

Notes:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from ADI's IFU may yield inaccurate results.
10.All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11.It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

REAGENTS PREPARATION FOR THE ASSAY:

Wash Buffer (50X): Dilute the wash buffer with distilled or deionised water (dissolve content of **1 bottle (20 ml) into 980 ml water**). Some buffer components may crystallize in wash concentrate. These redissolve at room temperature. Store diluted wash buffer at 2-8oC.

Sample Buffer (5x): Dilute the sample buffer with distilled or deionised water (dissolve content of **1 bottle (20 ml) into 80 ml water**).

Preparation of Samples: Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. Do not freeze and thaw. Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Quality Control:

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE, 25-28oC, BEFORE USE). Prepare working solutions of sample buffer and wash buffer (see page 3). Bring all reagents and solutions to room temp. (25-28oC).*

1. Organize the microplates' wells for standard, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette **100 µl** of ready-to-use standards, controls and prediluted patient samples into the wells. Incubate for **30 minutes** at room temperature (25-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of 1x wash solution. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.
4. Add **100 µl** of enzyme conjugate into each well and Incubate for **15 minutes** at room temperature.
5. Discard the contents of the microwells and wash 3 times with 300 µl of 1x wash solution.
6. Add **100 µl of TMB substrate** reagent to all wells. Note: Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.
7. **Incubate at room temperature for 15 minutes**.
8. Add **100 µl of stop solution** to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
9. **Read the absorbance in each well at 450nm** (using a reference wavelength of 620-630 nm. The results should be read within fifteen (15) minutes of adding the stop solution.

Note:
Positive control Range: 360-640 IU/ml;
Negative Control Range: <50 IU/ml