THERATEST LABS
Innovations in Immunoassays

EL-Anti-TPO™

An enzyme immunoassay for the
qualitative detection of IgG class antibodies against thyroid peroxidase (TPO) in human serum

For professional use only

Instruction Manual

Catalog No.: 104-119

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INTRODUCTION

Intended use

For in vitro diagnostic use

The **TheraTest EL-Anti-TPO™** is an enzyme immunoassay for qualitative detection with numerical expression of IgG class antibodies against thyroid peroxidase (TPO) in human serum. The **TheraTest EL-Anti-TPO™** assay is intended for use as an aid in the diagnosis of autoimmune thyroid disorders in conjunction with other clinical findings and laboratory tests.

Summary and Explanation

Autoimmune thyroid diseases include Hashimoto's thyroiditis, Graves' disease and various other conditions, like postpartum, silent or subclinical thyroiditis. Thyroid peroxidase (TPO) and thyroglobulin are important autoantigens involved in the pathogenesis of autoimmune thyroid diseases, and autoantibodies directed against these molecules are important aids for diagnosis (1, 2). TPO is a membrane-bound glycoprotein enzyme of 107 kDa in the thyroid tissue that liberates iodine for addition onto tyrosine residues of thyroglobulin for the production of thyroxine (T_4) or triiodothyronine (T_3).

Autoimmune thyroid disease, based on the data of comprehensive studies, is a frequent condition (3-6). Its prevalence is higher in women than in men, and increases with age. In the US population, hypothyroidism was found in 4.6% of more than 17,000 individuals (0.3% clinical and 4.3% subclinical), while hyperthyroidism was found in 1.3% of the study subjects (0.5% clinical and 0.7% subclinical) (5).

Anti-TPO antibodies are present in ~95% of patients with Hashimoto's thyroiditis and silent thyroiditis, and are also present in ~85% of patients with Graves' disease (1, 2). However, anti-TPO antibodies are relatively frequent (10-12%) in the disease free population (3-6). Their occurrence is more common in women than in men, and among the elderly. The presence of anti-TPO antibodies is a risk factor for developing autoimmune thyroid disease (1, 6-8). In anti-TPO positive patients, thyroid clinical examination and TSH determination could be recommended to detect subclinical thyroid dysfunction. Autoimmune thyroiditis is more prevalent in subjects with various autoimmune and non-autoimmune disorders and some genetic conditions. These include rheumatoid arthritis, diabetes mellitus, celiac disease, scleroderma, SLE, autoimmune polyglandular syndromes, Down syndrome, autoimmune liver diseases and pernicious anemia (2, 7, 9-13).

Principle of the procedure

The **TheraTest EL-Anti-TPO™ assay** is a solid phase enzyme immunoassay for the qualitative detection of anti-TPO antibodies. The wells of 96-well polystyrene plates have been coated with native human TPO antigen. The wells are incubated with a Calibrator, diluted serum specimens, Specimen Diluent Blank, and Controls. During the incubation, the antibodies present in the test sample bind to the solid phase antigen. Then the wells are washed, and horseradish-peroxidase labeled anti-human IgG (Fcγ specific) is incubated in the wells. Unbound anti-IgG antibody is removed by aspiration and washing. A specific chromogen substrate is added to the wells, and the autoantibody + anti-IgG complex is detected by a resulting color change, which is measured by a spectrophotometric enzyme immunoassay reader. A direct relationship exists between the

amount of anti-TPO antibodies in the specimen and the absorbance value detected by the spectrophotometer. Results are reported as U/ml (qualitative method with numerical expression) based on the value of the Calibrator provided.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only

Reagents Containing Human Source Material

Controls and Calibrators contain human serum. Treat as potentially infectious. When tested by FDA-cleared methods for the presence of antibody to HIV (Human Immunodeficiency Virus) and Hepatitis C Virus and for Hepatitis B Surface Antigen (HBsAg), the materials were nonreactive. While these methods are highly accurate, no test method can offer complete assurance that HIV, hepatitis virus or other infectious agents are absent. Therefore these materials and all patient specimens should be handled as though capable of transmitting infectious diseases. Human material should be handled in accordance with good laboratory practices using appropriate precautions as described in the Centers for Disease Control and Prevention/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories 5th Edition", 1 December 2009 (2009-12-01), pages 1 – 416. Web site: https://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

Stop Reagent (2 mol/L Phosphoric Acid)

Corrosive! May cause severe burns upon contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amount of water for at least 15 minutes.

Hazardous Substance Risk & Safety Phrases:

R34 - Causes burns.
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection
S45 - In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).

Chromogen

Irritant! This product contains 3,3',5,5'-tetramethylbenzidine (TMB) (≤0.05%), a chromogenic indicator of horseradish peroxidase activity. It has shown neither mutagenic nor carcinogenic effects in laboratory experiments (14).

Hazardous Substance Risk & Safety Phrases:

R36/37/38 – Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.
S24/25 – Avoid contact with skin or eyes.
S26 – In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36 – Wear suitable protective clothing.
S51 – Use only in well-ventilated areas.

Reagents Containing Sodium Azide

Calibrator and Controls contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush drain with large quantities of water to prevent azide build-up.

Hazardous Substance Risk & Safety Phrases:

R22 - Harmful if swallowed.
R36/37/38 - Irritating to eyes, respiratory system, and skin. Avoid inhalation and direct contact.
S26 - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S28 - After contact with skin, wash immediately with plenty of water.
S36/37/39 - Wear suitable protective clothing, gloves and eye/face protection
S46 - If swallowed, seek medical advice immediately and show this container label.

General Precautions and Information

1. Do not pipette by mouth.
2. Do not eat, drink, or smoke in designated work areas.
3. Wash hands thoroughly after using specimens and kit reagents.
4. Do not use test components beyond the expiration date.
5. Work in a well ventilated area when using kit reagents.
6. Avoid exposing reagents to excessive heat or light during storage.
7. Do not allow the Chromogen to come in contact with metal or oxidizing agents.
8. Use disposable glassware and plasticware or wash all material thoroughly according to standard laboratory practice.
9. Calibrators and Controls are lot specific and therefore are not interchangeable among kits of different lot numbers.
10. Avoid microbial contamination of the reagents.
11. Dispose of containers and unused kit reagents in accordance with local regulatory requirements

STORAGE AND HANDLING

1. Store all reagents at 2 – 8ºC when received. Avoid freezing reagents.
2. All reagents must be brought to room temperature (18 – 25ºC) for 30 minutes prior to use.
3. Avoid direct sunlight.
4. **Important**: When stored at 2 – 8ºC, the 10X Wash Buffer may form crystals. The crystals must be dissolved prior to dilution of the 10X concentrate when only a portion of the concentrate is being diluted. If all of the bottled contents are transferred at once to a 1-L graduated cylinder, be sure to rinse the bottle multiple times with water to dissolve and transfer any crystallized salts. When stored at 2 – 8ºC, the 10X Wash Buffer is stable until kit expiration, the 1X Wash Buffer is stable for 8 weeks.

SPECIMEN REQUIREMENTS

Collection and Storage of Serum

A whole blood specimen should be obtained using accepted medical techniques to avoid hemolysis. The blood should be clotted and the serum separated by centrifugation within 24 hrs of collection. Grossly hemolyzed, lipemic or icteric serum is not acceptable, since it may affect the results of the test. Serum may be stored at 2- 8 °C for up to 7 days. If testing cannot be completed within 7 days of collection, the separated serum must be stored at –20°C. Do not use serum that has been thawed more than once or which has been heat inactivated. The performance of plasma samples has not been evaluated; therefore plasma should not be used in the test.

PROCEDURE

Before starting the assay, read the product insert carefully. Instructions should be followed exactly as they appear in this kit insert to ensure valid results.

Materials Provided

1. **Two plates of antigen coated wells in 96-well plate format:** For single use only**!** All wells are coated with TPO antigen. Wells are printed with the name of the antigen. The unused wells and the frame may be stored and used at a later date. They are returned to their desiccant-containing pouch, which is sealed and stored dry at 2 - 8°C until the expiration date.
2. **10X Wash Buffer, 100 mL:** 10X concentrated buffer with preservative.
3. **TPO/Thyroglobulin Specimen Diluent 2x 115 mL**: Buffer with bovine protein and preservative.
4. **TPO Calibrator, 2x 1.5 mL each:** Calibrators contain human serum with various concentrations of IgG antibodies to TPO and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
5. **Positive Control, 0.35 mL:** Human serum containing IgG antibodies to TPO and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
6. **Negative Control, 0.35 mL:** Human serum without IgG antibodies to TPO and preservative in stabilizing buffer. See attached Data Sheet for performance characteristics.
7. **Anti-TPO Enzyme Conjugate, 27 mL:** Goat anti-human IgG (Fcγ specific) conjugated with horseradish peroxidase, with preservative in stabilizing buffer and green dye.
8. **Chromogen, 27 mL:** 3,3'5,5' tetramethybenzidine (TMB) in buffer with hydrogen peroxide.
9. **Stop Reagent, 27 mL:** 2 mol/L phosphoric acid.
10. **Re-sealable pouch**.

Materials required but not provided

1. Calibrated precision micropipettes with disposable plastic tips that deliver 5 µL, 100 µL and 1 mL.
2. Calibrated adjustable multichannel pipettes (8- or 12-channel).
3. Disposable Pipette tips.
4. Microtubes, polypropylene (dilution tubes or cluster tubes) with a rack of 96-well format.
5. Timer.
6. Pipettes (1 mL, 5 mL, and 10 mL).
7. Pipette reagent reservoirs (to accommodate multichannel pipettes).
8. Deionized or distilled water.
9. Single (450 nm) or dual (450 nm test, 620-690 nm reference) wavelength spectrophotometer (ELISA plate reader) for 96-well microtiter plates.
10. Clean wash bottle and automated plate washer (optional).

Reagent preparation:

1. Coated Wells

A suggested plate arrangement of wells is shown on the attached Data Sheet. The entire plate or strip (or strips) may be employed, or individual wells may be used as desired.

2. Wash Solution

The 10X Wash Buffer must be diluted 1:10 with deionized or distilled water prior to use. Prepare 1X Wash Buffer by pouring the contents of the 10X Wash Buffer into a clean one liter volumetric container. Rinse the bottle with deionized or distilled water to remove residual buffer and redissolve any existing crystals. Add the rinse to the one liter container. Add deionized or distilled water until a total volume of 1.0 L is reached; mix thoroughly. Diluted Wash Buffer is stable for 8 weeks at 2 - 8 °C.

3. Specimens, Positive Control, **Negative Control**

Specimens and Controls must be diluted 1:201 in the provided TPO/Thyroglobulin Specimen Diluent prior to being tested. Use high accuracy pipettes. For example, pipette 5 µL of serum into 1 mL of specimen diluent. Discard any unused diluted Specimens and Controls after the test procedure is completed.

4. Calibrator

Calibrators are provided pre-diluted, ready to use.

Assay Procedure

1. Allow all reagents and patient sera to equilibrate to room temperature prior to use (18 - 25°C). Plates should equilibrate to room temperature in their sealed foil pouch to prevent condensation.
2. Mark the position of the samples (i.e., Calibrator, Positive Control, Negative Control, Specimen Diluent Blank, and Specimens) on a worksheet, and arrange dilution tubes accordingly in a rack. A suggested plate arrangement is shown on attached Data Sheet.
3. Determine the number of wells needed. The remaining unused wells should be returned and resealed in the pouch with desiccant for later use.
4. Dispense 1 mL of TPO/Thyroglobulin Specimen Diluent into each dilution tube.
5. Dilute all serum Specimens and Controls 1:201 (e.g. add 5 µL of serum to 1 mL TPO/Thyroglobulin Specimen Diluent) and mix well. Do not dilute Calibrator.
6. Pipette 100 µL of the Calibrator, diluted samples, Specimen Diluent Blank, and diluted Controls into the appropriate wells. For best results pipette all materials within 5 minutes from the start of the assay. This step is facilitated by the use of multichannel pipettes.
7. Incubate the plate for 30 - 35 minutes at room temperature (18 - 25 °C).
8. Aspirate or decant the contents of the wells and wash the plate 3 times with 300 µL of 1X Wash Buffer. An automated plate washer may be used for this step. Remove all residual liquid from the wells by inverting and blotting the plate on absorbent paper.
9. Immediately pipette 100 µL of IgG Enzyme Conjugate into the wells.
10. Incubate plate(s) for 30 - 35 minutes at room temperature (18 - 25 °C).
11. Aspirate or decant Enzyme Conjugate from all wells and wash the plate as in Step 8 above.
12. Immediately dispense 100 µL of Chromogen into each well. Incubate the plate(s) for 15(±1) min. at room temperature (18 - 25 °C).
13. Pipette 100 µL of Stop Reagent into each well and mix by gently tapping the side of the plate. The blue color changes to yellow.
14. Determine the absorbance of each well at 450 nm using a single or dual wavelength spectrophotometer (ELISA plate reader). Absorbance values should be read within 30 minutes of completing the assay. For a dual wavelength spectrophotometer, set test wavelength at 450 nm with the reference between 620 and 690 nm.

Procedural Notes

1. Storage
Place unused strips in the open metallized pouch (with desiccant) for light protection and place this assembly into the provided re-sealable pouch and store at 2-8 °C.

2. Pipetting
To avoid cross-contamination and sample carryover, pipette the Calibrator, Positive Control, Negative Control, Specimen Diluent Blank, and Specimens using separate pipette tips. A multi-channel pipette may be used to pipette the Enzyme Conjugate, Wash Solution, Chromogen, and Stop Reagent.

3. Washing
Each column of wells may be washed using a multi-channel pipette. The wells may be aspirated using an appropriate vacuum apparatus, fitted with a Pasteur pipette, or their contents may be dumped into a disposal container. Alternatively, commercial semi-automated washing systems may be used. When using either washing technique, the plate should be inverted and blotted against absorbent paper after the last wash. Use reagent grade water only (CAP type 1 or USP grade) for preparing the 1X Wash Buffer.

4. Measurement of Absorbance Values
Absorbance values should be measured within 30 minutes after completion of the assay.

RESULTS

Calculation of Results
Most ELISA readers are computer compatible and data may be calculated with the help of computer programs. Check periodically that the program chosen yields the same results as obtained by manual calculations.

The specific absorbance (net absorbance values) for samples, Calibrator and Controls are calculated by subtracting the absorbance value of Specimen Diluent Blank from the absorbance value of the corresponding well.

EXAMPLE:

Absorbance for Specimen Diluent Blank = 0.050

Absorbance for Specimen well = 1.150

Net absorbance for the Specimen is 1.150 – 0.50 = 1.100

Note: If the absorbance value of Specimen Diluent **B**lank is higher than that of the Specimen, the net absorbance should be considered zero.

Antibody activity is calculated as follows:

$$\text{Conversion Factor} = \frac{\text{Units/mL value of TPO Calibrator}}{\text{Net absorbance (OD) value of TPO Calibrator}}$$

Antibody Units/mL in Specimen = Conversion Factor x Net absorbance value of Specimen

Interpretation
Reference range/negative: 0-5 U/mL
Equivocal range: 6-8 U/mL
Positive: ≥ 9 U/mL

The above ranges are suggested values only. The reference range should be validated by each laboratory to reflect the characteristics of the population they serve. When the results are equivocal it is recommended to report them as equivocal, and repeat the test 3-6 months later.

QUALITY CONTROL

1. Positive and Negative Controls

Positive and Negative Controls should be run in each assay. The Controls should be tested as unknowns. The Positive and Negative Control values should fall within the ranges provided on the enclosed lot specific Data Sheet. If the values are not in agreement with those on the Data Sheet, the assay is not valid and the results should not be reported.

LIMITATIONS OF THE PROCEDURE

1. The Positive Control and the Calibrator for a specific antibody may contain other antibodies, i.e. they may not be monospecific.
2. The **TheraTest EL-Anti-TPO™** assay should not be performed on grossly hemolyzed, lipemic, icteric or microbially contaminated samples. The effect of hemolysis, lipemia, and icterus has not been evaluated with this assay.
3. This method has been tested using serum samples only. The performance using other types of specimens has not been determined.
4. Diagnosis should not be made solely on the basis of a positive test result. The results must be interpreted in conjunction with all clinical information and laboratory data available to the physician (i.e. history, physical exam, and other diagnostic procedures).
5. This assay has not been evaluated on a pediatric population.
6. Equivocal results are suggested to be repeated 3-6 month later, in conjunction with thyroid function tests.
7. If the absorbance value of the Specimen exceeds the linear range of the reader, the result should be reported as > U/mL (of the upper limit of the linear range). If endpoint result is desired, the Specimen should be pre-diluted (example: 1:10) with the provided TPO/Thyroglobulin Specimen Diluent, and the Specimen should be retested. The retest result should be multiplied by the pre-assay dilution factor (for example, if the Specimen was pre-diluted 1:10, the units obtained should be multiply by 10). There is no linear relationship between the dilution factor and the obtained unit values.

EXPECTED VALUES

Anti-TPO antibodies are present in ~95% of patients with Hashimoto's thyroiditis and silent thyroiditis, and are also present in ~85% of patients with Graves' disease (1, 2). The expected value in the normal population is negative. However, anti-TPO antibodies are relatively frequent (10-12%) in the disease free population (3-6). Their occurrence is more common in women than in men, and also in the older population. The presence of anti-TPO antibodies is a risk factor for developing autoimmune thyroid disease (1, 6-8). Autoimmune thyroiditis is more prevalent in subjects with various autoimmune disorders including rheumatoid arthritis, diabetes mellitus, celiac disease, scleroderma, SLE, autoimmune polyglandular syndromes, autoimmune liver diseases, pernicious anemia (2, 7, 9-13).

PERFORMANCE CHARACTERISTICS

The Clinical Sensitivity and specificity of this assay has not been assessed.

Comparative studies

A total of 109 samples were tested by the **TheraTest EL-Anti-TPO™** assay and another commercially available anti-TPO immunoassay. The results are presented in the table below:

TheraTest EL-Anti-TPO™ *versus another anti-TPO immunoassay (n=109)*

		Other Anti-TPO assay		
		Positive	**Negative**	**Total**
EL-Anti-TPO™	**Positive**	62	0	62
	Negative	4	41	45
	Equivocal	2	0	2
	Total	68	41	109

Agreement (equivocal results considered as positive):
Percent Positive Agreement: (64/68)*100 = 94.1% (95% CI: 85.6% to 98.4%)
Percent Negative Agreement: (41/41)*100 = 100.0% (95% CI: 91.4% to 100.0%)
Percent Total Agreement: (105/109)*100 = 96.3% (95% CI: 90.9%-99.0%)

Agreement (equivocal results considered as negative):
Percent Positive Agreement: (62/68)*100 = 91.2% (95% CI: 81.8% to 96.7%)
Percent Negative Agreement: (41/41)*100 = 100.0% (95% CI: 91.4% to 100.0%)
Percent Total Agreement: (103/109)*100 = 94.5% (95% CI: 88.4%-98.0%)

Interference

Potential interference from autoimmune disease specimens was evaluated in a representative study by testing specimens with known autoantibody positivity. Specimens containing anti-TPO antibodies of 133.6 and 1085.3 IU/mL were spiked with sera containing anti-dsDNA antibodies (450 IU/mL), anti-MPO antibodies (115 U/mL) and anti-CCP antibodies (119 U/mL). Mean absolute recovery is summarized in the table below:

Autoantibody	*% Recovery of anti-TPO antibody concentration*
Anti-dsDNA	94.3-105.5
Anti-MPO	100.6-102.7
Anti-CCP	90.3-101.0

Precision

Two specimens with different levels of reactivity were tested 20 times within the same respective assay (within-run precision/repeatability) and 20 different times in one or two runs per day (between-run/total precision). The obtained precision results are presented in the following table:

Within-run precision/repeatability		**Between-run/total precision**	
mean (U/mL)	%CV	mean (U/mL)	%CV
6.7	3.1	5.7	11.1
51.9	1.8	48.3	5.2

Limit of detection

The limit of detection of the **TheraTest EL-Anti-TPO™** assay was calculated to be 1.2 U/mL.

High concentration hook effect

High dose hook effect is a phenomenon whereby specimens containing extremely high concentration of analyte produce false negative or low values. For the **TheraTest EL-Anti-TPO™** assay no hook effect was observed when samples containing up to approximately 16,000 IU/mL of anti-TPO antibodies were tested.

TROUBLESHOOTING

Problem	Possible Causes	Solution
Control values out of range.	1. Incorrect temperature, timing or pipetting; reagents not mixed.	1. Check that temperature was correct. Check that time was correct. See "Poor Precision" (below) No. 2-4. Repeat test.
	2. Cross-contamination of controls.	2. Pipette carefully.
	3. Improper dilution.	3. Repeat test.
	4. Optical pathway not clean.	4. Check for moisture or dirt. Wipe bottom and reread.
	5.Wavelength of filter incorrect.	5. Change filter to 450 ± 5 nm.
All test results negative.	1. One or more reagents not added, or added in wrong sequence.	1. Recheck procedure. Check for unused solutions. Repeat test.
	2. Improper dilution of wash buffer.	2. Repeat test.
	3. Antigen coated plate inactive.	3. Check for obvious moisture in unused wells. Rerun test with controls only for activity.
All test results yellow.	1. Contaminated chromogen.	1. Check absorbance of unused chromogen.
Scattered false positives	2. Contaminated buffers/reagents.	2. Check all solutions for turbidity.
	3. 1X Wash Buffer contaminated.	3. Use clean container. Check quality of water used to prepare buffer.
	4. Improper dilution of serum.	4. Repeat test.
	5. Contaminated pipette	5. Use felt-plugged tips for chromogen
Poor precision.	1. Pipettor delivery CV greater than 5%.	1. Check calibration of pipettor. Use reproducible technique.
	2. Serum or reagents not mixed sufficiently; reagents not at room temperature prior to addition.	2. Mix all reagents gently but thoroughly and equilibrate to room temperature.
	3. Reagent addition taking too long; inconsistency in timing intervals, air bubbles.	3. Develop consistent uniform technique and avoid splashing or use multi-channel device or autodispenser to decrease time.
	4. Air currents blowing over plate during incubations.	4. Cover plate or place in chamber.
	5. Optical pathway not clean.	5. Wipe bottom of plate with soft tissue. Check instrument light source and detector for dirt.
	6. Instrument not equilibrated before readings were taken.	6. Check instrument manual for warm up procedure.
	7. Washing not consistent; trapped bubbles; liquid left in wells at end of wash cycle.	7. Use only acceptable washing devices. Lengthen timing delay on washing devices. Check that all wells are filled.
	8. Improper pipetting.	8. Avoid air bubbles in pipette tips.

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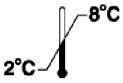
(GB)(USA)(CDN) Manufactured by (D)(A)(B)(CH) Hergestellt von (F)(B)(CH)(CDN) Fabriqué par (I)(CH) Prodotto da (E) Fabricado por (P) Fabricado por (NL) Vervaardigd door (DK) Fabrikation af (S) Tillverkad av

(GB)(USA)(CDN) Catalogue Number (D)(A)(B)(CH) Bestell-Nummer (F)(B)(CH)(CDN) Numéro de référence (I)(CH) Numero di riferimento (E) Número de referencia (P) Número de referência (NL) Referentienummer (DK) Referencenummer (S) Katalognummer



(GB)(USA)(CDN) Store at between (D)(A)(B)(CH) Lagerung bei zwischen (F)(B)(CH)(CDN) Conserver à entre (I)(CH) Conservare a tra (E) Conservar a temp. entre (P) Armazene a entre (NL) Bewaar bij tussen (DK) Opbevares mellem (S) Förvaras vid

(GB)(USA)(CDN) Contains sufficient for x tests (D)(A)(B)(CH) Inhalt ausreichend für x Tests (F)(B)(CH)(CDN) Contient suffisant pour x tests (I)(CH) Contenuto sufficiente per x test (E) Contiene suficiente para x pruebas (P) Contém suficiente para x testes (NL) Bevat voldoende voor x bepalingen (DK) Indeholder tilstrækkeligt til x prøver (S) Innehållet räcker till x analyser

(GB)(USA)(CDN) Caution, Consult accompanying documents. (D)(A)(B)(CH) Achtung. begleitdokumente beathten. (F)(B)(CH)(CDN) Attention, consulter les documents joints. (I)(CH) Attenzione, consultare la documentazione allegata. (E) Precaucion, consultar la documentacion adjunta. (P) Cuidado, consulte a documentação fornecida. (NL) Let op, raadpleeg bijgeleverde documenpen. (DK) Forsigtig, Læs ledsagende dokunenter. (S) Forsiktig, se vedlagt dokumentasjon.

Abbreviated Test Procedure

1. **Dilute Controls and Specimens 1:201 with TPO/Thyroglobulin Specimen Diluent.**
2. **Pipette 100 µL of Calibrator, diluted Controls, Specimen Diluent Blank, and Specimens into appropriate wells (see Data Sheet for configuration).**
3. **Incubate for 30-35 minutes at room temperature (18º - 25ºC).**
4. **Wash the wells three times with 1X Wash Buffer.**
5. **Add 100 µL of the Anti-IgG Enzyme Conjugate into appropriate wells.**
6. **Incubate for 30-35 minutes at room temperature (18º - 25ºC).**
7. **Wash the wells three times with 1X Wash Buffer.**
8. **Add 100 µL of Chromogen into each well.**
9. **Incubate for 15±1 minutes at room temperature (18º- 25ºC).**
10. **Add 100 µL of Stop Reagent into each well.**
11. **Read the absorbance at 450 nm (reference wavelength 620-690 nm) within 30 minutes.**

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