DRG® TPO IgG ELISA (EIA-2791)

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IVD

INTENDED USE

The DRG TPO IgG ELISA test system is intended for the qualitative and semi-quantitative detection of IgG-class antibody to thyroid peroxidase (TPO) in human serum. The test system is intended to be used as an aid in the diagnosis of thyroid diseases. This test is for *in vitro* diagnostic use only.

SIGNIFICANCE AND BACKGROUND

Thyroid antibodies are a characteristic finding in patients with Hashimoto's and Graves' diseases (1). The presence of thyroid antibodies in the sera of 80% of patients with these two diseases led to the recommendation that some type of thyroid antibody testing be a feature of the work-up of any patient with a goiter (1). Although thyroid antibodies are predominantly associated with Hashimoto's or Graves' diseases, they may be found in the sera of patients with other diseases such as myxedma, granulomatous thyroiditis, nontoxic nodular goiter, and thyroid carcinoma (1). Thyroid antibodies are also found in most cases of lymphocytic thyroiditis in children (2), and rarely in patients with pernicious anemia and Sjogren's Syndrome (3-4).

PRINCIPLE OF THE ELISA ASSAY

The DRG Thyroid Peroxidase (TPO) ELISA test system is designed to detect IgG class antibodies to TPO in human sera. Wells of plastic microwell strips are sensitized by passive absorption with TPO antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG ***(y*** chain specific) is added to the wells and the plate is incubated. The Conjugate will react with the antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

MATERIALS PROVIDED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

1. Plate. 96 wells configured in twelve 1x8-well strips coated with purified human thyroid peroxidase {> 98% pure). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG (V chain specific). Ready to use. One. 15 mL vial with a white cap.
3. Positive Control (Human Serum). One. 0.35 mL vial with a red cap.
4. Calibrator A (Human Serum). One, 0.35 mL vial with a white cap.
5. Calibrator B (Human Serum). One. 0.35 mL vial with a yellow cap.
6. Calibrator C (Human Serum). One, 0.35 mL vial with an orange cap.
7. Calibrator D (Human Serum). One, 0.35 mL vial with a blue cap.
8. Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
9. SAVe Diluent"* (Sample Diluent). One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 ± 0.2). Ready to use. **Note: Shake Well Before Use**. **NOTE: The SAVe Diluent'" will change color in the presence of serum.**
10. TMB: One 15 mL amber botlle (amber cap) containing **3.3'.5, 5'**-tetramethylbenzidine (TMB). Ready to use. Contains DMSO < 15% (w).
11. Stop solution: One 15 mL bottle (red cap) containing 1**M** H 2 SO 4, 0.7M HCI Ready to use.
12. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 ± 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

Note: Kit also contains:

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

PRECAUTIONS

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS.** Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg. and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodbome Pathogens (5).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20-25°C) before starting the assay.** Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe diluent™, controls, wash buffer, and conjugate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.

9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green or very pale blue when used. Contamination of the TMB with conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

MATERIALS REQUIRED BUT NOT PROVIDED:

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200^L.
- Multichannel pipette capable of accurately delivering (50-200/**JL)**
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water. « One liter graduated cylinder. « Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant, (example: 10% household bleach, 0.5% sodium hypochlorite.)

STORAGE CONDITIONS

1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
7. SAVe Diluent™: Store between ***2°*** and 8°C.
8. Stop Solution: Store between ***2°*** and 25°C.

SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (6, 7). No anticoagulants or preservatives should be added. Avoid using hemoiyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

GENERAL PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow seven Control/Calibrator determinations (one Blank, one Negative Control, four Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 2
B	Neg. Control	Patient 3
C	Calibrator A	Etc.
D	Calibrator B	
E	Calibrator C	
F	Calibrator D	
G	Pos. Control	
H	Patient 1	

3. Prepare a 1:21 dilution (e.g.: 10uL of serum + 200uL of SAVe Diluent™. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. The SAVe Diluent™ will undergo a color change confirming that the specimen has been combined with the diluent.
4. To individual wells, add 100uL. of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent™ to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25^{o}C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

A. Manual Wash Procedure:

a. Vigorously shake out the liquid from the wells.
b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
c. Repeat steps a. and b. for a total of 5 washes.
d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350uL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100/µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 + 5 minutes
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100/µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25X) for 10 to 15 minutes.
13. Stop the reaction by adding 50/µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

QUALITY CONTROL

1. Each time the assay is run, the positive control, negative control, calibrator A, B, C, and D must be included. Additionally, a reagent blank must be included.
2. The specifications for the positive and negative controls are as follows:.
   Positive Control Must be > 30 lU/mL
   Negative Control Must be < 15 lU/mL
   a. The OD of the negative control divided by the OD of the positive control should be ≤0.9.
   b. If the control values are not within the above ranges, the test should be considered invalid and the test should be repeated
3. The positive control is intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
4. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

1. Calibrator

Based upon testing of normal sera, disease state sera, and the World Health Organization (WHO) international standard (66/387), a maximum normal lU/mL value has been determined by the manufacturer and correlated to the calibrators. The calibrators will allow you to determine the unit value for each of the test samples evaluated. The unit values are determined for each lot of kit produced, and are printed on the Component List included with each kit.

2. Quality Control

Refer to the specification sheet included with each kit. This sheet describes the lot specific specifications for each of the calibrators. If any of the calibrators are out of range, the results are considered invalid, and the patient results may not be reported.

3. Conversion of Optical Density to lU/mL

Optical densities of the specimens are determined from the standard curve generated from the calibrators. A standard curve should be generated using the paired data points for each of the four calibrators (OD on the Y axis and corresponding lU/mL value on the X axis). Using the best fit, point to point curve, and determine the lU/mL value for each of the specimens tested by extrapolation.

LIMITATION OF THE ASSAY

1. A diagnosis should not be made solely on the basis of the ELISA result. Test results for anti-thyroglobulin should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Reproducible results with an ELISA system require careful pipetting, strict adherence to incubation periods and temperatures requirements, as well as thorough washing of the test wells and thorough-mixing of all solutions.
3. Hemolytic, icteric, or lipemic samples may interfere with this ELISA. Use of these types of specimens should be avoided.

EXPECTED RESULTS

The clinical investigation included 80 random normal donor specimens. With respect to this group, four (5%) were positive and 76 (95%) were negative.

PERFORMANCE CHARACTERISTICS

Comparative Study:

An in-house comparative study was performed to determine the equivalence of the DRG TPO IgG ELISA test system to another commercially available TPO IgG ELISA test system. Performance was evaluated using 248 specimens and the results have been summarized in Table 1 below.

Table 1. Summary of the comparative investigation, DRG® ELISA test system versus a commercially available ELISA test system.

DRG TPO IgG ELISA Test System

		Pos.	Equivocal*	Neg.	Totals
Commercial TPOIgG ELISA Test System	**Positive**	104	12	7	123
	Negative	7	1	117	125
	Totals	111	13	124	248

* Equivocal specimens were excluded from calculations below.

Relative* Sensitivity =	104 /111	= 93.7 %	- 95% Confidence Interval of 89.1 to 98.2%
Relative Specificity =	117 /124	= 94.4 % -	95% Confidence Interval of 90.3 to 98.4%
Relative Agreement =	221 /235	= 94.0 %	■ 95% Confidence Interval of 91.0 to 97.1%

* Please be advised that the term 'relative' refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.

Reproducibility

A study was conducted in-house to determine reproducibility. Briefly, six specimens were tested; two negative, two strong positive, and two positive specimens that were near the assay cut off. Each specimen was tested in eight replicate wells on each day, for a total of three days. The resulting data was used to determine both intra and inter assay reproducibility. A summary of the study appears in Table 2 below.

INTERPRETATION OF RESULTS:

Using normal healthy individuals,disease-state specimens, and the WHO standard, the manufacturer has establishedthe following guidelines for interpretation of patient results:

< 25 lU/mL	Negative
25-30 lU/mL	Equivocal*
> 30 lU/mL	Positive

* Specimens which are repeatedly equivocal should be tested using an alternate serological method.

Cross Reactivity.

To evaluate the system for potential cross reactivity to other autoantibodies, seventeen specimens which were positive for antibodies to nuclear antigens (ANA) on HEp-2 cells were tested. Of the specimens tested, none were positive on the TPO IgG ELISA. This study indicates that the potential for interference due to cross reactive autoantibodies is unlikely.

Correlation to the World Health Standard (N1BSC 66/387)
The World Health Standard (NIBSC 66/387) was tested on the DRG assay to determine the correlation of the result obtained to the expected result. The data from this study in presented in Table 3 below.

Dilution of the Standard	IU/mL as Tested	OD (450 nm)	Result: (IU/mL)
neat	1000	> 3.000	278
1:2	500	> 3.000	275
1:4	250	2.710	243
1:8	125	1.740	144
1:16	62	0.890	61
1:32	32	0.457	30
1:64	16	0.202	14
1:128	8	0.112	8

REFERENCES:

1. Beall GN, Solomon DH: Post. Grad. Med. 54:181, 1973.
2. Tung KS, Ramos CV, Deodhar SD: Am. J. Clin. Pathol. 61:549, 1974.
3. Beall GN, Solomon DH: Ed Samter, 2nd Edition, Boston, Little, Brown and Company, pp. 1198-1213, 1971.
4. Doniach D, and Roitt IM: Clin. Immunol. 2nd Edition (Ed) Gell PH, and Coombs RRA, Oxford, Blackwell, Chapter 35, 1968.
5. U.S. Department of Labor, Occupational Safety and Health Administration: Occupational Exposure to Bloodborne Pathogens Final Rule. Fed. Register 56:64175-64182, 1991.
6. Procedures for the collection of diagnostic blood specimens by venipuncture - Second edition. Approved Standard (1984). Published by National Committee for Clinical Laboratory Standards.
7. Procedures for the Handling and Processing of blood Specimens. NCCLS Document H18-A, Vol. 10, No. 12, Approved Guideline, 1990.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100/uL/well
3. ---------- ► *Incubate 20 to 30 minutes*
4. Wash
5. Add Conjugate - 100 /µL/well
6. ---------- ► *Incubate 20 to 30 minutes*
7. Wash
8. Add TMB 100 *ul/well*
9. ----------► *Incubate 10 to 15 minutes*
10. Add Stop Solution 50 µL/well - Mix
11. READ