

THYROGLOBULIN ANTIBODY II

REF A32898

Intended Use The Access Thyroglobulin Antibody II (TgAb) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin antibody levels in human serum and plasma using the Access Immunoassay Systems. The measurement of thyroid autoantibodies may aid in the diagnosis of Hashimoto's disease, nontoxic goiter, and Graves' disease.

Summary and Explanation Thyroglobulin is produced by the thyroid gland. It is a water soluble glycoprotein of approximately 660,000 Daltons. It is a major component of the thyroid follicular colloid and is present in small amounts in serum. The principal role of thyroglobulin is the storage and synthesis of thyroid hormones. The thyroid hormones 3, 5, 3', 5', - tetraiodothyronine (thyroxine, T4) and 3, 5, 3', -triiodothyronine (T3) are synthesized from thyroglobulin.¹ Thyroglobulin autoantibodies (TgAb) are often present in patients with autoimmune thyroid disease. Approximately 10% of healthy individuals have TgAb at measurable levels. TgAb can be detected in 30% of patients with Graves' disease and in 85% of patients with Hashimoto's thyroiditis.² However, elevated levels of autoantibodies to thyroid peroxidase (TPO autoantibodies) occur more frequently than high TgAb levels in these diseases. Sensitive TgAb methods are needed to identify patient sera that contain thyroglobulin autoantibodies that may interfere with serum thyroglobulin measurements.

Principles of the Procedure The Access Thyroglobulin Antibody II assay is a sequential two-step immunoenzymatic ("sandwich") assay. A sample is added to a reaction vessel with paramagnetic particles coated with the thyroglobulin protein. The serum or plasma TgAb binds to the thyroglobulin. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The thyroglobulin-alkaline phosphatase conjugate is added and binds to the TgAb. After the second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of thyroglobulin antibody in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information **Access Thyroglobulin Antibody II Reagent Pack**
Cat. No. A32898: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 56 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

R1a:	Dynabeads** paramagnetic particles coated with streptavidin and coupled to biotinylated human thyroglobulin, suspended in a TRIS buffer with protein (bovine), < 0.1% sodium azide, and 0.1% ProClin*** 300.
R1b:	Human thyroglobulin-alkaline phosphatase (bovine) conjugate in a TRIS buffer with protein (bovine), < 0.1% sodium azide, and 0.1% ProClin 300.
R1c:	TRIS buffer with < 0.1% sodium azide and 0.1% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.³
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.⁴
- Xi. Irritant: 0.1% ProClin 300.



R 43: May cause sensitization by skin contact.
 S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin/EDTA) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:⁵
 - Collect all blood samples observing routine precautions for venipuncture.
 - Allow serum samples to clot completely before centrifugation.
 - Keep tubes stoppered at all times.
 - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
 - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
 - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
 - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
3. Use the following guidelines when preparing specimens:
 - Ensure residual fibrin and cellular matter has been removed prior to analysis.
 - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
5. Thaw samples no more than two times.

Materials Provided R1 Access Thyroglobulin Antibody II Reagent Packs

Materials Required But Not Provided

1. Access Thyroglobulin Antibody II Calibrators
Provided at zero and approximately 50, 250, 500, 1000 and 2500 IU/mL.
Cat. No. A36920
2. Control (QC) materials: commercial control material.
3. Access Substrate
Cat. No. 81906
4. Access Wash Buffer
Cat. No. 81907 (Access, Access 2, SYNCHRON LX[®]i)
Cat. No. 8547197 (UniCel[®] DxI)

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use ten (10) μ L of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is IU/mL.

Procedure Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results

Calibration Details An active calibration curve is required for all tests. For the Access Thyroglobulin Antibody II assay, calibration is required every 56 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.⁶ Include commercially available quality control materials that cover at least two levels of analyte. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results Patient test results are determined automatically by the system software using a smoothing spline math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

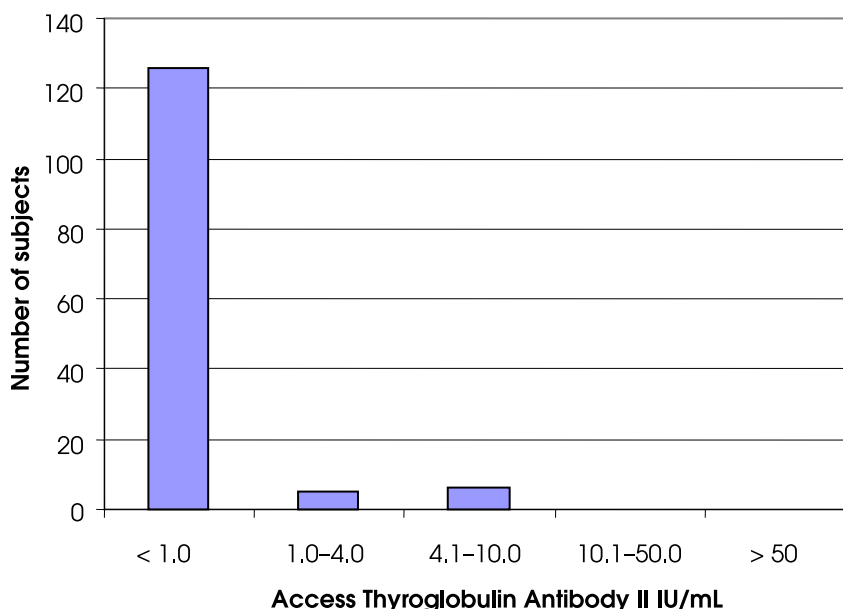
Limitations of the Procedure

1. Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.9–2500 IU/mL).
 - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.9 IU/mL).
 - If a sample contains more than the stated value of the highest Access Thyroglobulin Antibody II Calibrator (S5), report the result as greater than that value (i.e., > 2500 IU/mL). Alternatively, dilute one volume of sample with 9 volumes of Access Thyroglobulin Antibody II Calibrator S0 (zero). Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.^{7,8}
Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Thyroglobulin Antibody II results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
4. The Access Thyroglobulin Antibody II assay does not demonstrate any “hook” effect up to approximately 350,000 IU/mL.

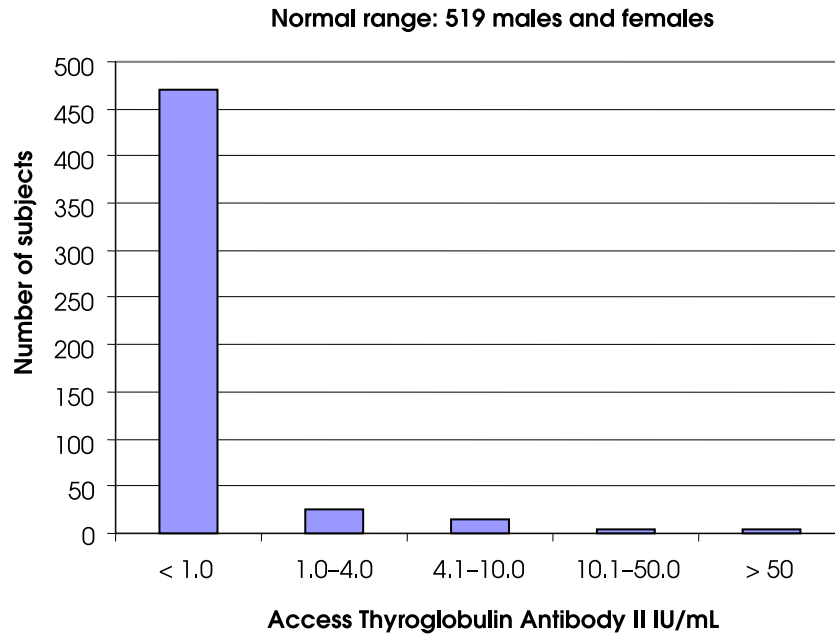
Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Sera samples were obtained in the United States from males < 30 years of age following the criteria outlined by the National Academy of Clinical Biochemists (NACB) for establishing a normal reference range for thyroid antibody tests.⁹ The screening criteria included serum TSH levels between 0.5 and 2.0 mIU/L, no personal or family history of thyroid disease and absence of non-thyroid autoimmune disease. 137 screened samples were tested generating a 95% non-parametric upper reference limit below 4 IU/mL.

Normal range: 137 males < 30 years old



3. Additionally, 519 normal samples were collected in the United States from both males and females ranging in age from 18–74 years old. The screening criteria included serum TSH levels between 0.5 and 2.0 mIU/L no personal or family history of thyroid disease, and absence of non-thyroid autoimmune disease. 519 samples were tested. 96% of these samples fell below 4 IU/mL.



**Specific
Performance
Characteristics**

Methods Comparison

A comparison of 832 values using the Access Thyroglobulin Antibody II assay on the Access Immunoassay system and a commercially available enzyme immunoassay kit gave the following results using relative sensitivity, specificity and percent agreement:

		Commercially Available TgAb Assay			
		+	-	Total	
Access Thyroglobulin Antibody II Assay	+	136	3	139	Positive % Agreement = 95.1%
	-	7	686	693	Negative % Agreement = 99.6%
	Total	143	689	832	Percent Agreement = 98.8%

Demographics

Subject Category	Number of Subjects	Gender	Age Mean (years)	Age Range (years)
Normal	531	262 Females 269 Males	35	18–74
Subjects with Graves' disease	112	58 Females 54 Males	52	14–95
Subjects with Hashimoto's Disease	106	72 Females 33 Males 1 Unknown	59	22–89
Subjects with thyroid cancer	19	17 Females 2 Males	50	33–75
Subjects with anemia	10	8 Females 2 Males	64	40–96
Subjects with type 1 diabetes	10	6 Females 4 Males	70	37–91
Subjects with elevated thyroid antibody test	44	21 Females 3 Males 20 Unknown	51	2–83

Dilution Recovery (Linearity)

Gravimetric dilution of three samples containing various TgAb levels with Access Thyroglobulin Antibody II Calibrator S0 (zero) resulted in the following data:

Note: Due to varying antigen specificity, affinity and avidity of thyroglobulin antibodies in their epitope reactions some samples may not dilute linearly.¹⁰

Sample 1	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	1497.5	1497.5	100
1:1.33	1123.1	1109.7	99
1:2	748.7	816.6	109
1:4	374.4	469.6	125
1:8	187.2	269.1	144
1:16	93.6	150.2	160
		Mean % Recovery	127

Sample 2	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	168.6	168.6	100
1:1.33	126.5	121.0	96
1:2	84.3	97.9	116
1:4	42.2	53.7	127
1:8	21.1	26.6	126
1:16	10.5	13.5	128
		Mean % Recovery	119

Sample 3	Expected Concentration (IU/mL)	Determined Concentration (IU/mL)	Recovery (%)
Neat	808.6	808.6	100
1:1.33	606.4	588.2	97
1:2	404.3	422.2	104
1:4	202.1	255.7	126
1:8	101.1	132.4	131
1:16	50.5	67.2	133
		Mean % Recovery	118

Imprecision

This assay exhibits total imprecision of less than 10% CV for concentrations greater than or equal to 15 IU/mL and < 1.5 IU/mL SD at concentrations < 15 IU/mL. One study, using commercially available human serum based control material generating a total of 40 assays, 3 replicates per assay, over 20 days provided the following data, analyzed via analysis of variance (ANOVA).¹¹

Patient Sample	Mean (IU/mL)	Within Run (% CV)	Between Run (% CV)	Total Imprecision (% CV)
1	27.0	5.8	3.1	6.6
2	147.8	4.2	4.5	6.2
3	151.2	4.0	2.7	4.8
4	329.7	4.6	3.3	5.7
5	338.7	4.1	3.5	5.4
6	721.0	4.9	5.1	7.0

Patient Sample	Mean (IU/mL)	SD
1	1.7	0.3
2	4.0	0.4
3	7.2	0.5
4	10.5	0.8

Analytical Specificity / Interferences

Thyroglobulin antibody concentrations of >12 IU/mL do not have significant interference at the following interferent concentrations.

Substance	Interferent Added
Bilirubin	40 mg/dL
Triglycerides (Triolein)	3000 mg/dL
Total protein (human serum albumin)	6 g/dL
Hemoglobin	500 mg/dL

To assess the potential for autoimmune disease interference various disease state samples were tested with a predicate thyroglobulin antibody device. Total agreement of 97% was achieved (28/29). The following table describes the results.

Other autoimmune disease/conditions	n
ANA	5
aDNA	5
RA	5
RF	10
SLE	4
Total	29

Autoantibody % Agreement

Commercially Available TgAb Assay					
Access Thyroglobulin Antibody II Assay		+	-	Total	
	+	1	0	1	
	-	1	27	28	
	Total	2	27	29	Percent Agreement = 97%

Analytical Sensitivity

The lowest detectable level of TgAb distinguishable from zero (Access Thyroglobulin Antibody II Calibrator S0) with 95% confidence is 0.9 IU/mL. This value is determined by processing a complete six point calibration curve, controls and 20 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is calculated from the curve at the point that is two standard deviations from the mean measured zero calibrator signal.

THYROGLOBULIN ANTIBODY II CALIBRATORS

REF A36920

Intended Use The Access Thyroglobulin Antibody II (TgAb) Calibrators are intended to calibrate the Access Thyroglobulin Antibody II assay for the quantitative determination of thyroglobulin antibody levels in human serum and plasma using the Access Immunoassay Systems. The measurement of thyroid autoantibodies may aid in the diagnosis of Hashimoto's disease, nontoxic goiter, and Graves' disease.

Summary and Explanation Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability The measurand (analyte) in the Access Thyroglobulin Antibody II Calibrators is traceable to the WHO 65/93 International Standard. Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information **Access Thyroglobulin Antibody II Calibrators**
Cat. No. A36920: S0, 4.0 mL/vial; S1–S5, 2.5 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card for exact concentrations.

S0:	Human serum with < 0.1% sodium azide and 0.5% ProClin*** 300. Contains 0.0 IU/mL thyroglobulin antibody.
S1, S2, S3, S4, S5:	Human thyroglobulin antibody in human serum at levels of approximately 50, 250, 500, 1000, and 2500 IU/mL, respectively, with < 0.1% sodium azide and 0.5% ProClin 300.
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.³

- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.⁴
- Xi. Irritant: 0.5% ProClin 300.



R 43: May cause sensitization by skin contact.

S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Material Safety Data Sheet (MSDS) is available upon request.
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Procedure Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details The Access Thyroglobulin Antibody II Calibrators are provided at six levels – zero and approximately 50, 250, 500, 1000 and 2500 IU/mL. Assay calibration data are valid up to 56 days.

Calibrators run in duplicate.

Limitations of the Procedure If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

References

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