



UltraPlex™ THYROID

80 samples | 60 TESTS

A multiplexed immunoassay for the
quantitative detection of
autoantibodies to TPO and TG.

FOR IN VITRO DIAGNOSTIC USE



PRONOSTICS
Knowledge for Health

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UltraPlex™ Thyroid for 80 samples (160 tests)

For in vitro diagnostic use only. Store at 2-8°C upon receipt.

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Intended use

The UltraPlex™ Thyroid assay is intended for use in the quantitation of TPO (Thyroid Peroxidase) and TG (Thyroglobulin) autoantibodies in human serum.

Summary and explanation of the assay

In thyroid autoimmune conditions, the patient generates antibodies against thyroid proteins and hormones. These autoantibodies alter the delicate balance of thyroid hormone production which in turn leads to significant changes to the body metabolism rate. The major autoimmune thyroid diseases are: Hashimoto's Thyroiditis, Primary Hypothyroidism (Myxoedema) and Graves' disease. The relative levels of TPO and TG autoantibodies are indicators of disease state. The test results are not diagnostic but are one aspect of diagnostic analysis of individuals. The UltraPlex™ Thyroid assay is a multiplexed, barcoded microparticle immunoassay for the quantitative detection of autoantibodies TPO and TG in human serum in one simple assay. The barcoded microparticles provide a solid phase for the immunoassays. Each set of barcoded microparticles has a unique autoantigen bound to its surface. The UltraPlex™ SmartStation performs all liquid handling, incubation, tracking of samples and pipettes the completed assay particles into a read plate. The UltraPlex™ SmartReader detects, identifies and measures the fluorescence of each barcoded microparticle, enabling the two immunoassays to be performed simultaneously on a serum sample in a single well.

Principle of the UltraPlex™ Thyroid assay

The UltraPlex™ Thyroid test system contains pre-formulated SmartBeads (1 per microwell). Each SmartBead contains the two autoantigens (TPO and TG), each of which is bound to a distinct UltraCode (barcoded microparticle). Details of the antigens are given in Appendix 1.

In addition to the two autoantigens, a third control UltraCode with no antigen coating (Qmax zero) is also present. This enables the monitoring of non-specific binding of samples to the UltraCodes.

Diluted serum samples are incubated with the SmartBeads to allow any antibodies present in the serum to bind to the antigens. After washing away unbound antibodies, the bound antibodies are detected using anti-human IgG fluorescent conjugate. After incubation, excess detection antibody is washed away. The UltraPlex™ SmartReader detects the fluorescence associated with each UltraCode and gives a quantitative result for each individual immunoassay (50-500 IU/ml or 50-500 U/ml range for TG or TPO respectively).

Reagents and materials supplied

THYROID ASSAY PLATE One 96 well filter bottom microplate containing 1 SmartBead per well and sealed in an aluminium bag with a drying agent. Ready to use.

SAMPLE DILUTION BUFFER (5x) One 20 ml bottle of concentrated solution, containing a detergent and protein in phosphate buffered saline with preservative. Dilute 1 in 5 to a final volume of 100 ml.

WASH BUFFER (10x) One 27.5 ml bottle of concentrated solution, containing a detergent in phosphate buffered saline with preservative. Dilute 1 in 10 to a final volume of 275 ml.

STANDARDS Six vials each containing 300 μ l of diluted human serum. The expected results are given on the control certificate. Ready to use.

NEGATIVE CONTROL One vial containing 300 μ l diluted normal human serum. The expected results are given on the control certificate. Ready to use.

POSITIVE CONTROL 1 One vial containing 300 μ l of diluted human serum. The expected results are given on the control certificate. Ready to use.

DETECTION ANTIBODY One 15 ml bottle of anti-human IgG dye conjugate in protein-stabilising matrix with a preservative. Ready to use.

DECODING BUFFER One 25 ml bottle of buffer containing glycerol in phosphate buffered saline with a preservative. Ready to use.

READING PLATE One 96 well clear bottom black microtitre plate.

BATCH ID TUBE One vial displaying the batch record information.

Storage and stability

Store the kit at 2–8°C in the original container until use. The reagents are stable until the expiration date on the kit. Do not expose kit to heat, sun or strong light during storage or usage. Do not freeze.

Materials and equipment required

1. Distilled or de-ionised water and clean measuring cylinders for the dilution of buffers.
2. UltraPlex™ SmartReader.
3. UltraPlex™ SmartStation.
4. One 96 deep well plate (Sarstedt Cat# 82.1970.002).
5. Four boxes Rainin 1000 ml tips (Anachem Cat# RT-L1000).
6. Plate sealing tape.

Warnings and precautions

1. For *in vitro* diagnostic use only.
2. Not for internal use in humans or animals.
3. Not to be used as a standalone product in making diagnosis, monitoring or screening decisions. The result of this test must not be used for determination of medical intervention.
4. The controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, these reagents should be handled as per local regulations.
5. Optimal results will be obtained by strict adherence to the test protocol.
6. Avoid contact of reagents and samples with the eyes, skin and clothing. Wear disposable gloves, lab clothing and eye protection while handling samples and reagents. Wash hands thoroughly when finished. If contact occurs, wash thoroughly with water or at eyewash stations.
7. The components of this kit are intended for use as an integral unit. The components of different units should not be mixed.
8. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
9. Do not use kit components past the stated expiry dates. Avoid microbial contamination of all reagents and samples or incorrect results may occur.
10. The procedure is for running 80 test samples simultaneously rather than in small batches.

Specimen collection and handling

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. If frozen, ensure samples are thoroughly defrosted and mixed before use.
3. Avoid repetitive freezing and thawing of serum sample. Lipaemic, haemolysed or contaminated samples may give poor results and should not be tested.
4. A minimum sample volume of 100 µl is required for the UltraPlex™ SmartStation.
5. Up to 80 serum samples can be tested per run.
6. Samples must be contained in tubes compatible with use with the UltraPlex™ SmartStation sample racks.

Preparation for the assay

1. Bring all specimens and kit reagents to room temperature (18-25°C) and gently mix.
2. Dilute contents of wash buffer concentrate 10x to 275 ml with distilled or de-ionised water in a suitable storage container.
3. Dilute contents of sample dilution buffer concentrate 5x to 100 ml with distilled or de-ionised water in a suitable storage container.
4. Briefly invert to mix and then centrifuge the tubes containing the standards and controls to ensure the liquid is at the bottom of the tubes.
5. Ensure that the samples are fully mixed, especially if they have been stored for a long period of time.
6. It is important to not introduce air bubbles into either the controls or samples during any mixing.

UltraPlex™ Thyroid assay procedure

The assay procedure is composed of two elements. Firstly, the UltraPlex™ SmartStation robotic liquid handling system performs all liquid handling, incubation, tracking of samples and then pipettes the completed assay particles into a read plate. Secondly, the UltraPlex™ SmartReader detects, identifies and measures the fluorescence of each UltraCode and provides the data output.

Separate operating manuals describe the operation of the UltraPlex™ SmartStation and SmartReader, and should be read thoroughly in conjunction with this user guide. Those operating manuals describe in detail the set-up, maintenance and shut-down procedures of the UltraPlex™ SmartStation and SmartReader.

The aspects of the running of the UltraPlex™ SmartStation that are specific to the UltraPlex™ Thyroid assay are described below:

1. The UltraPlex™ Thyroid assay is designed to test 80 samples on a single assay plate. Load these samples into positions 1 – 80 of the barcode cassettes.
2. Load the controls and standards provided onto the sixth barcode cassette of the UltraPlex™ SmartStation in the following order:
 1. Negative control
 2. Positive control 1
 3. Standard 1
 4. Standard 2
 5. Standard 3
 6. Standard 4
 7. Standard 5
 8. Standard 6

3. Load the batch number tube in position 89 of the barcode cassette.
4. Load the barcode cassettes onto the UltraPlex™ SmartStation deck in the order cassette 1 to cassette 6 from left to right.
5. Load the reagent racks as follows:
 1. 50 ml of diluted sample dilution buffer
 2. 50 ml of diluted sample dilution buffer
 3. All of provided detection antibody (15 ml)
 4. All of provided decoding buffer (25 ml)

The remainder of the set-up of the UltraPlex™ SmartStation is not unique to the UltraPlex™ Thyroid assay, and is detailed in the operating instructions for the UltraPlex™ SmartStation. For convenience they are also provided in summary form below and in the quick start guide.

1. Place diluted wash buffer in the wash buffer trough.
2. Load four full tip boxes on the deck.
3. Place a fresh piece of blotting paper on the blotting block.
4. Load the deep well dilution plate, read plate and assay plate onto the deck in their respective positions, with well A1 in the top left in each case.

The assay can now be started using the UltraPlex™ Thyroid assay file in the Lirix software, as described in the UltraPlex™ SmartStation operating manual.

Following completion of the liquid handling steps, seal the UltraPlex™ Thyroid read plate with a plate sealing tape and transfer to the UltraPlex™ SmartReader system for detection, identification and measurement of the fluorescence of each UltraCode. This procedure is detailed in the UltraPlex™ SmartReader operating manual.

Interpretation of the results

The UltraPlex™ SmartDecode software returns a quantitative result for TG in IU/ml and TPO in U/ml using a separate standard curve for each. The TG assay is calibrated in IU/ml against the 1st international reference preparation (NIBSC code 65/093)¹ defined as 1000 IU/ml. The TPO assay is calibrated against the NIBSC research standard (NIBSC code 66/387)², defined as 5000 U/ml

From studies of samples from 400 healthy blood donors and 400 clinical samples, characterized by alternative commercial ELISAs, Pronostics have established the following guidelines for interpretation of the data:

	Anti-TG autoantibody (IU/ml)	Anti-TPO autoantibody (U/ml)
Normal	<150	<75
Borderline	150-175	75-100
Elevated	>175	>100

Quality control

In order for the assay to be valid, all the following criteria must be met:

- Standards and positive and negative controls must be included in each assay run.
- The values obtained for all the controls should be in the ranges specified on the QC certificate
- The curve shape should be similar to the standard curve shown in the QC certificate

If any of the above criteria are not met, the assay is invalid and should be repeated.

Performance characteristics

Precision

Intra-assay precision (within an assay) was calculated for each sample from the results of twenty replicates in a single assay.

Inter-assay precision (between assays) was calculated for each sample from the results of three different assays with twenty replicates of each sample on each assay.

The results shown are calculated results for TG (IU/ml) and TPO (U/ml).

Antigen	CV (%)
TG	18.7
TPO	13.8

Table 1. Intra-assay precision data

Antigen	CV (%)
TG	12.4
TPO	19.3

Table 2. Inter-assay precision data

Linearity of the assays

To assess the linearity of the TG and TPO assays, four samples, containing ranges of concentrations of anti-TG and anti-TPO autoantibodies were serially diluted with sample dilution buffer to produce a range of values within the dynamic range of the assay.

The samples showed linearity across the full range of the assay, with dilutions of the four samples from 1:200 to 1:12800. The mean of observed over expected values is 95% (range 78 to 125%) for TG and 103% (76 - 127%) for TPO.

Sensitivity of the assay

Sensitivity was calculated from the combined results of two studies. Firstly, eight replicates of each of two serum samples (negative for anti-TG and anti-TPO autoantibodies) were analysed in a single assay. Secondly, 400 samples from healthy donors were analysed for their levels of anti-TG and anti-TPO autoantibodies.

The sensitivity of the assay was determined to be <50 IU/ml TG and <50 U/ml TPO.

Warranty

The UltraPlex™ Thyroid assay is warranted to perform as described in this packaging insert. Any change or modification to the procedure as described by Pronostics Ltd may affect the results and is not recommended. Pronostics Ltd disclaims any implied warranty of merchantability or fitness for a particular use, and in no event will Pronostics Ltd. be liable for consequential or indirect damages.

References

1. WHO Expert Committee on Biological Standardization, 30th Report. WHO Technical Report Series No. 638 (1979).
2. WHO Expert Committee on Biological Standardization, 29th Report. WHO Technical Report Series No. 262 (1978)

Company information

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Appendix 1. Details of autoantigens

Antigen	Description	Purity
Thyroglobulin (TG)	Native, isolated from human thyroid glands	>90 % by SDS-PAGE
Thyroid peroxidase (TPO)	Recombinant*	>90 % by SDS-PAGE

* Expressed by recombinant baculovirus infection of *Spodoptera frugiperda* Sf9 insect cells.

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