11. Assay Procedure Continued…

9. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

Controls are intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD-values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

Plot the OD of each standard against its concentration and draw the best-fit curve through the points. Read the unknowns off this curve. In normal individuals, circulating Tg values range from 2 – 50 ng/ml.

Values above 400 ng/ml should be repeated at a dilution of 1:5 in sample diluent.

14. Limitations of the Procedure

1. Results must be interpreted in conjunction with other clinical information relating to the patient.
2. Samples that contain thyroglobulin autoantibodies may produce misleading results and should be interpreted with caution.

15. Performance Characteristics

Assay Sensitivity
0.6ng/ml

16. Reproducibility

Within Assay Precision
CV%: Typically 5 – 10%

Between Assay Precision
CV%: Typically 8 -20%

17. Method Summary

- Dispense 50µl of each standard, control and sample into the microplate wells
- Dispense 50µl of Sample Diluent into all wells except those containing the 0 U/ml standard.
- Incubate for **60 minutes** at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **30 minutes**
- *Wash the wells four times*
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10 minutes**
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm
- 450/620nm (dual wavelength).

18. Further Reading

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G·E·N·E·S·I·S Diagnostics

Thyroglobulin Antigen ELISA Kit

Quantitative assay for the detection of human thyroglobulin antigen

Product Code: GD101

For *in vitro* Diagnostic Use

CE

1. Materials Included in the Kit

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with rabbit anti-thyroglobulin antibodies, with holder in a foil bag with desiccant.
- **Reagent 1**: **Sample diluent** 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent, 10ml, (blue), ready to use
- **Reagent 2: Wash buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 75 ml, **concentrate** (X 10)
- **Reagent 3: Conjugate** anti-human thyroglobulin IgG conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, (red) ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standards:** 10, 50, 100, 200, 400 ng/ml, 1ml of 10mM Tris-buffered saline containing human thyroglobulin, ready to use
- **Elevated control:** 1ml of 10mM Tris-buffered saline containing thyroglobulin, (green), ready to use
- **Normal control:** 1ml of 10mM Tris-buffered saline containing normal human serum, (turquoise), ready to use
- **Instructions for use**

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 50µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use

The Thyroglobulin Antigen Kit is a rapid ELISA method for the detection of human thyroglobulin. It is intended as an aid to the diagnosis of thyroid disease. The components of the kit are for *in vitro* diagnostic use only.

4. Explanation of the Test

Thyroglobulin (Tg) is a glycoprotein of approximately 650,000 Daltons, which is synthesized and stored in the follicular colloid of the thyroid gland. It functions as a pro-hormone during the synthesis of thyroxine (T4) and triiodothyronine (T3).

Only thyroid-derived tissues produce Tg. Normal individuals have circulating Tg levels between 2-50 ng/ml. As functioning thyroid tissue is the only source of circulating Tg, the measurement of this pro-hormone in thyroidectomized individuals (via surgery and with or without radio-iodine ablation) is useful clinically as an aid to the detection of local and metastatic thyroid tissue. Accordingly, serum Tg determinations are often widely used to complement other diagnostic aids, such as whole-body scan, computed tomography, high-resolution ultrasound, and chest x-ray, to detect the presence of functional thyroid tissue or tumour.

Elevated serum concentrations of Tg have been reported in various thyroid diseases, such as hyperthyroidism, non-toxic goiter, thyroiditis, and differentiated thyroid carcinoma.

5. Principle of the Test

Serum specimens are incubated for 60 minutes to allow Tg to bind to anti-Tg antibody-coated wells. After washing away unbound serum constituents, bound Tg is detected using rabbit anti-human Tg conjugated to horseradish peroxidase. After 30 minutes incubation, unbound conjugate is removed by washing, and TMB Substrate is added. A blue colour develops in Tg-positive wells. Addition of Stop Solution gives a yellow colour and the optical densities of standards, controls and samples are measured using a microplate reader.

6. Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 - 8ºC after use.
3. Include the Positive Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and positive control into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 - 8ºC.

9. Specimen Collection and Storage

Fresh serum or plasma samples should be used in the assay. If not being tested within 48 hours, samples should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

Dilute the Wash Buffer **(Reagent 2)** 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

11. Assay Procedure

1. Assemble the number of strips required for the assay.

2. Dispense 100 µl of sample diluent as the 0 U/ml standard. Dispense 50 µl each standard, control or patient sample into the wells. Dispense 50 µl of sample diluent into all wells except those containing the 0 U/ml standard.

3. Incubate for **60 minutes** at room temperature.

4. After 60 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   <u>Manual Wash Procedure</u>
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with Wash Buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

5. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **30 minutes** at room temperature.

6. After 30 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.

7. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **10 minutes**.

8. Add 100µl of Stop Solution (**Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.