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**Date:** 10/12/04

**Ref:** 167/MKT/E/52

**SUBJECT** **Product launching**

**We are happy to announce a new family of products on the bioelisa product range:**

**3000-1145 bioelisa TSH 96 Tests**
**3000-1146 bioelisa fT3 96 Tests**
**3000-1147 bioelisa fT4 96 Tests**
**3000-1150 bioelisa anti-Tg 96 Tests**
**3000-1151 bioelisa anti-TPO 96 Tests**

**These assays are designed for the quantitative determination of the thyroid hormones and auto-antibodies affecting the thyroid function.**

**Find enclosed product information that we hope that it will help you in the introduction of these new assays.**

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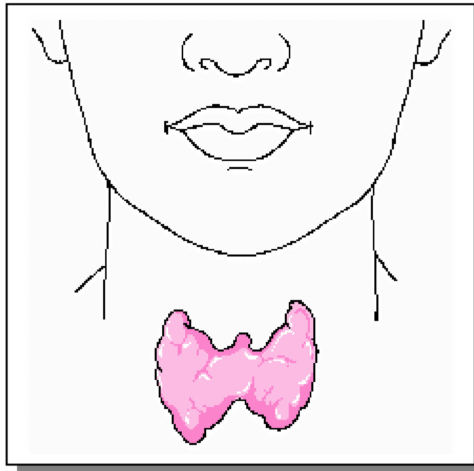
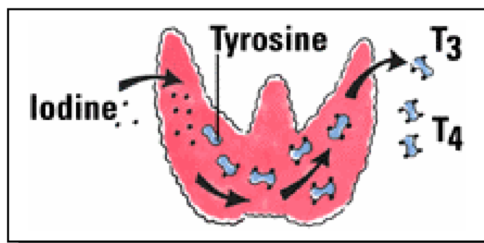
# Background on Thyroids:

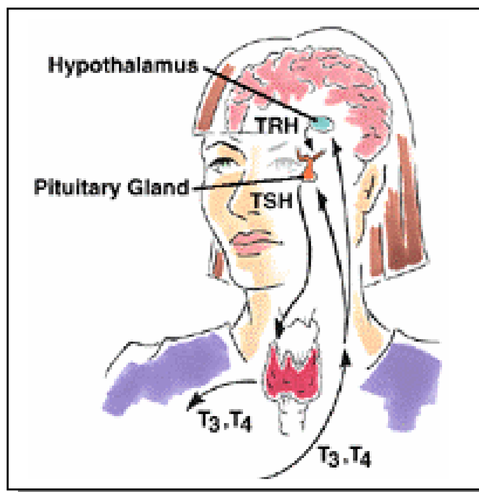


## The thyroid function

The thyroid gland is the largest pure endocrine gland in the body, and is located on the trachea just inferior to the larynx (Adam's apple). The major role of the thyroid gland is the production of TH or thyroid hormone, which is the hormone that controls the metabolic rate of our body and is what controls the body's temperature. There are two active iodine-containing hormones, thyroxine, (**T4**) and triiodothyronine (**T3**).




The function of the thyroid gland is to take iodine, found in many foods, and convert it into thyroid hormones: thyroxine (T4) and triiodothyronine (T3). These hormones are then released into the blood stream and are transported throughout the body where they control metabolism (conversion of oxygen and calories to energy). Every cell in the body depends upon thyroid hormones for regulation. The normal thyroid gland produces about 80% T4 and about 20% T3; however, T3 is about four times as strong as T4.




The thyroid gland is under the control of the pituitary gland, a small gland the size of a peanut at the base of the brain. When the level of thyroid hormones drops too low, the pituitary gland produces THYROID STIMULATING HORMONE (**TSH**), which stimulates the thyroid gland to produce more hormones. This raises the blood level of T4 and T3, and the pituitary TSH level then decreases. Free hormones (**fT3** and **fT4**) are generally considered to provide the more reliable indication of true thyroid status because only the free hormone is physiologically active. The total (but not the free) hormone concentration is dependent on the concentration of thyroid transport proteins, specifically thyroid binding globulin (TBG), which is influenced by many common factors.

One can imagine the thyroid gland as a furnace and the pituitary gland as the thermostat. Thyroid hormones are like heat. When the heat gets back to the thermostat, it turns the thermostat off. As the room cools (the thyroid hormone levels drop), the thermostat turns back on (TSH increases) and the furnace produces more heat (thyroid hormones).

The pituitary gland itself is regulated by another gland, known as the hypothalamus. The hypothalamus is part of the brain and produces TSH Releasing Hormone (TRH) which tells the pituitary gland to stimulate the thyroid

gland (release TSH). One might imagine the hypothalamus as the person who regulates the thermostat since it tells the pituitary gland at what level the thyroid should be set.

## Thyroid diseases

Thyroid disorders are among the most common endocrinological diseases.

Various things may go wrong with the thyroid gland. It may produce too little thyroid hormone (hypothyroid). It may produce too much thyroid hormone (hyperthyroid). Hypothyroidism can reduce the metabolic rate to as low as 1/2 the normal rate, whereas hyperthyroidism can possibly double it.

The thyroid gland may become inflamed (thyroiditis) or enlarged (goiter). It may develop a cyst, a benign growth, or malignant growth.

The causes of most types of thyroid disease remain largely unknown. A careful history will reveal a relative with a thyroid problem in approximately 50% of patients. Sometimes this may be a grandparent or an aunt so that it may seem to "skip" a generation. Various types of thyroid disease are sometimes seen in the same family. All types of thyroid disease are more common in women than in men.

**Hypotyroidism**

Hypothyroidism occurs when there are not enough of the thyroid hormones, T4 and T3, circulating in the blood. This disorder may also be described as an underactive thyroid. It is the most common form of thyroid dysfunction and affects approximately two out of 100 people. More women than men have underactive thyroid glands.

**Signs and symptoms**

Thyroid hormone influences how fast the body produces and uses energy; an insufficient level of thyroid hormone slows down the body. For example, the brain, heart, blood vessels, liver, muscles, skin, and reproductive system all require thyroid hormone to function normally. If the level of thyroid hormone in the blood is too low, these parts of the body can begin to function poorly and produce a variety of signs and symptoms:

| | |
|---|---|
| • fatigue | • drowsiness |
| • feeling cold | • difficulty concentrating |
| • poor memory | • feeling "blue" |
| • difficulty losing weight | • loss of appetite |
| • dry, itchy skin | • hair loss |
| • dry, brittle hair | • brittle nails |
| • puffiness around the eyes | • hoarseness |
| • muscle pain and cramps | • constipation |
| • an enlarged thyroid gland (goiter) | • slow heart rate |
| • slow reflexes | • elevated cholesterol |

| • heavy menstrual periods | • loss of interest in sex |
|---|---|

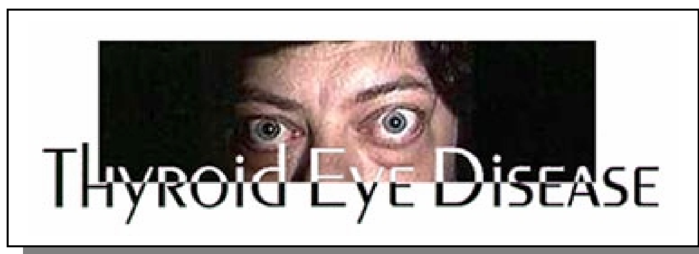
The type and severity of the signs and symptoms vary from patient to patient. Hypothyroidism can develop slowly, over time, and some patients may be unaware of any symptoms during the first stages of the disease. On the other hand, those patients who do not feel well will often describe symptoms such as feeling tired and sleepy, being cold all the time, losing their hair, being depressed, having difficulty concentrating, and finding it difficult to lose weight.

**Diagnosis**

Since the signs and symptoms of hypothyroidism can also be the signs and symptoms of other, unrelated diseases, it is important to be examined by the physician and to perform appropriate laboratory tests. The most precise and accurate way to make a diagnosis of hypothyroidism is to measure blood concentrations of TSH and the thyroid hormone T4. A low level of T4 and a high TSH will confirm the diagnosis.

**Hyperthyroidism**

Hyperthyroidism occurs when too much thyroid hormone circulates in the blood. This disorder may also be described as an overactive thyroid. Of the approximately 2.5 million Americans who have hyperthyroidism, there are more women than men.




**Signs and Symptoms**

Thyroid hormone influences how fast the body produces and uses energy. For example, your brain, heart, blood vessels, liver, muscles, skin, and reproductive system all require thyroid hormone to function normally. If the level of thyroid hormone in your blood is too high, these parts of the body can become overstimulated and, over time, produce a variety of signs and symptoms.

| • nervousness | • irritability |
|---|---|
| • restlessness | • muscle weakness and fatigue |
| • shortness of breath | • feeling hot and sweaty |
| • difficulty sleeping | • heart palpitations |
| • abnormal heart rhythm | • trembling hands |
| • moist warm palms | • frequent bowel movements |
| • difficulty concentrating | • poor memory |
| • weight loss | • hair loss |
| • an enlarged thyroid gland | • drawn back eyelids |

The type and severity of the signs and symptoms vary from patient to patient. Typically, hyperthyroid patients describe feeling nervous and/or irritable, being hot and sweaty, having palpitations (irregular, fast, or strong heartbeat), having difficulty sleeping, experiencing muscle weakness and fatigue, and losing weight even though they may have an increased appetite. However, patients over 60 years of age may exhibit fewer and less obvious signs and symptoms.

**Graves' disease**

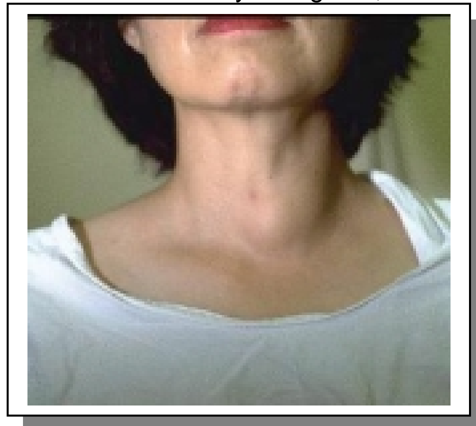
Graves' disease is the leading cause of hyperthyroidism in the United States. It is an autoimmune disorder that affects the thyroid gland, and may also involve the eyes, and the skin. Autoimmune diseases occur when the immune system produces antibodies against normal tissue in the body. In Graves' hyperthyroidism, thyroid stimulating antibodies attach to sites in the thyroid gland called TSH receptors and prompt the thyroid gland to make thyroid hormone. However, these antibodies are not under the control of the thyroid feedback mechanism and can overstimulate the thyroid gland, resulting in too much thyroid hormone.

**Diagnosis**

Since the signs and symptoms of hyperhyroidism can also be the signs and symptoms of other, unrelated diseases, it is important to be examined by the physician and to perform appropriate laboratory tests. Your physician will order tests to measure the amount of thyroid hormone and TSH in your bloodstream. A low TSH and a high level T4 and/or T3 indicate hyperthyroidism. Your physician may also check for the presence of thyroid stimulating antibodies, which are associated with Graves' disease. Checking your sedimentation rate can help determine if your hyperthyroidism is caused by subacute thyroiditis. Another indication of hyperthyroidism is an elevated thyroglobulin (thyroid hormones attach themselves to this substance).

**Goiters**

The term goiter means any enlargement of the thyroid gland. It refers only to the size of the thyroid gland, not to how well the thyroid gland is functioning. Therefore, a patient with a goiter may have too little, too much, or the proper amount of thyroid hormone. When the thyroid gland is uniformly enlarged, it is called a diffuse goiter. When a goiter contains one or more nodules, it is called a nodular goiter. A thyroid nodule is a localized growth in or on the thyroid gland. When discussing goiters and nodules, it is important to keep in mind that they are generally not cancerous.



**Signs and symptoms**

During the early stages of goiter development, most patients are unaware that they have a goiter. Symptoms can develop slowly over time as the goiter increases in size and presses on certain parts of the neck. Symptoms can include:

- Swelling in the neck, sometimes first noticed when trying to button a shirt
- Tight sensation in the throat
- Difficulty swallowing
- Hoarseness
- Impaired breathing

Patients with a goiter and an underactive thyroid gland that produces too little thyroid hormone can also have signs and symptoms of hypothyroidism, such as feeling tired and sleepy, being cold all the time, losing their hair, feeling "blue," having difficulty concentrating, and finding it difficult to lose weight.

Likewise, patients with a goiter and an overactive thyroid gland that produces too much thyroid hormone can also have the signs and symptoms of hyperthyroidism, such as being nervous and irritable, feeling hot all the time, sweating more than usual, having difficulty sleeping, experiencing heart palpitations, and losing weight.

**Causes**

The leading cause of goiters world-wide is a lack of iodine in the diet. Thyroid hormone contains molecules of iodine, and, when there is not enough dietary iodine, a goiter and/or hypothyroidism can develop. Iodine deficiency is rarely the cause of goiters in North America because iodine is added to salt and other foods. Instead, the most common causes of goiter are:

**High levels of TSH associated with hypothyroidism**

In addition to stimulating the thyroid gland to make thyroid hormone, TSH (thyroid stimulating hormone) causes the thyroid gland to grow. When there is not enough thyroid hormone in the body, the pituitary gland will increase production of TSH in an attempt to stimulate the thyroid gland to make more thyroid hormone. When patients are hypothyroid, their thyroid gland cannot make enough thyroid hormones, and the pituitary gland continues to produce TSH. Therefore, because of this continued elevation of TSH, the thyroid gland may enlarge.

**Antibodies associated with Graves' disease**

The antibodies associated with Graves' disease behave like TSH and stimulate the thyroid follicular cells to grow and produce thyroid hormone. However, production of these antibodies is not controlled by the normal thyroid feedback system. As a result, the thyroid gland is overstimulated and can become enlarged.

**Diagnosis**

During a physical examination, physicians will often begin by looking at the neck to see if it appears swollen. In order to determine if the thyroid gland is the source of the swelling, physicians may ask patient to swallow several times in order to observe or feel the movement of the thyroid gland. By gently pressing his or her fingers along the neck, the physician determines the size and texture of the thyroid gland as well as if there is tenderness. In some cases, he or she may detect the presence of one or more thyroid nodules.

Laboratory tests that measure the amount of thyroid hormone and TSH in the bloodstream will determine if the thyroid gland is functioning properly or if you are either hypo- or hyperthyroid. The physician may also order laboratory tests to measure thyroid antibodies that could be present with either Hashimoto's thyroiditis or Graves' disease.

Thyroid ultrasound can be an important diagnostic tool in evaluating goiters. This is a painless procedure that forms a picture of the thyroid gland by bouncing sound waves off of it. In addition to determining the size of a goiter, ultrasound can detect the presence of thyroid nodules as well as their size and structure. If the ultrasound demonstrates the presence of clinically significant nodules, then further tests will be necessary to determine whether the growth is cancerous.

## Thyroid autoimmune diseases.

Autoimmune diseases have been also associated to the Thyroid function and should be also investigated. Autoantibodies of clinical interest in thyroid disease include thyroid-stimulating antibodies (TSAb), TSH receptor-binding inhibitory immunoglobulins (TBII), antithyroglobulin antibodies (Anti-Tg) and the antithyroid peroxidase antibody (Anti-TPO). Of these, anti-TPO Ab has becomed as the most generally useful marker for the diagnosis and management of autoimmune thyroid disease.

Historically, **Anti-Tg** determinations were used in combination with antimicrosomal Ab determinations to enlarge the probability of a positive result in patients with autoimmune disease. Although the prevalence of Anti-Tg antibodies in thyroid autoimmune disease is high (85 percent and 30 percent in Hashimito's thyroiditis and Graves' disease, respectively), it is much lower than the prevalence of the Anti-TPO antibodies. The diagnostic utility provided by Anti-TPO assays is rarely improved upon by the addition of an Anti-Tg determination. The growing trend is to adopt the anti-TPO Ab test as the front-line test for autoimmune disease and no longer to routinely use the anti-Tg assay routinely for this purpose.

Because anti-Tg antibodioes constitute an interference in thyroglobulin (Tg) assays, another major use of the anti-Tg test is to screen samples that have been submitted for thyroglobulin determinations.

The **Anti-TPO** was historically referred to as the antimicrosomal antibody. The thyroid peroxidase enzyme (responsible for iodinating tyrosine residues in the thyroglobulin molecule) was subsequently identified as the major microsomal component recognized by these autoantibodies.

Anti-TPO antibodies affects antibody-dependent thyroid cell destruction; high levels correlate with the active phase of the disease. Measurement of this autoantibody is useful for resolving the diagnostic incoherence presented by the apparent inconsistency between elevated TSH and normal free T4 results. Given abnormally elevated TSH and euthyroid T4 results, a positive anti-TPO test provides strong evidence for early, subclinical autoimmune disease. Approximately 10 percent of asymptomatic individuals have elevated levels of Anti-TPO, which may suggest a predisposition to thyroid autoimmune disease. Elevated levels are found in virtually all cases of Hashimoto's thyroiditis and in approximately 85 percent of Graves' disease cases.

## Thyroid tests

T4 by RIA (radioimmunoassay), chemioluminiscence or enzymoimmunoassay are the most used thyroid test reflects the amount of thyroxine in the blood. If the patient does not take any type of thyroid medication, this test is usually a good measure of thyroid function. As stated earlier, thyroxine represents 80% of the thyroid hormone produced by the normal gland and generally represents the overall function of the gland. The other 20% is triiodothyronine measured as T3 by RIA. Sometimes the diseased thyroid gland will start producing very high levels of T3 but still produce normal levels of T4. Therefore measurement of both hormones provides an even more accurate evaluation of thyroid function.

Most of the thyroid hormones in the blood are attached to a protein called thyroid binding globulin (TBG). If there is an excess or deficiency of this protein it alters the fT4 or fT3 measurement but does not affect the action of the hormone. If a patient appears to have normal thyroid function, but an unexplained high or low

fT4, or fT3, it may be due to an increase or decrease of TBG. Direct measurement of TBG can be done and will explain the abnormal value.

Excess TBG or low levels of TBG are found in some families as an hereditary trait. It causes no problem except falsely elevating or lowering the T4 level. These people are frequently misdiagnosed as being hyperthyroid or hypothyroid, but they have no thyroid problem and need no treatment.

TSH is produced by the pituitary gland. Normally, low levels (less than 5 units) of TSH are sufficient to keep the normal thyroid gland. functioning properly.

When the thyroid gland becomes inefficient such as in early hypothyroidism, the TSH becomes elevated even though the fT4 and fT3 may still be within the "normal" range. This rise in TSH represents the pituitary gland's response to a drop in circulating thyroid hormone; it is usually the first indication of thyroid gland failure. Since TSH is normally low when the thyroid gland functions properly, the failure of TSH to rise when circulating thyroid hormones are low is an indication of impaired pituitary function. The new "sensitive" TSH test will show very low levels of TSH when the thyroid is overactive. Interpretations of the TSH level depends upon the level of thyroid hormone; therefore, the TSH is usually used in combination with other thyroid tests such as the fT4 and fT3. As fT4 and fT3, the TSH can be determined by RIA (radioimmunoassay), chemioluminiscence or enzymoimmunoassay.

Anti-Tg and anti-TPO determinations have been demonstrated very useful in resolving cases were Thyroid disease symptoms are observed and serological concentrations of TSH, fT3 and fT4 correspond to an euthyroid patient.

# bioelisa TSH

**ELISA test for the quantitative determination of Thyroid-stimulating hormone (TSH) in human serum or plasma.**

## Summary

Thyroid-stimulating hormone (TSH) is a glycopolypeptide hormone produced by the anterior pituitary gland in response to thyroid-releasing hormone (TRH) secreted by the hypothalamus. TSH stimulates the thyroid gland to synthesize and release the thyroid hormones, tri-iodothyronine (T3) and thyroxine (T4). Release of TSH is regulated by the circulating free fraction of thyroid hormones in the blood. TSH levels are depressed when peripheral concentrations of the free fraction of thyroid hormones are high. Conversely, TSH levels are high when peripheral concentrations of thyroid hormones are low. TSH values are important in the investigation and diagnosis of disorders of the thyroid gland and hypothalamic/pituitary axis. TSH assays are particularly useful in the differential diagnosis of hyperthyroid, euthyroid and hypothyroid patients.

## Principle

Monoclonal antibodies specific to human TSH molecule are immobilised on microplate wells and other antibodies to the TSH molecule are conjugated with biotin. TSH from the sample is bound to the well and biotin conjugate is added. After a washing step, streptavidin-HRP conjugate is added. After a second washing step, substrate is added. The enzymatic reaction is proportional to the amount of TSH in the sample. The reaction is terminated by adding stopping solution. Absorbance is measured on a plate reader.

## Components

1. MICROPLATE:
12 x 8 wells coated with anti-TSH monoclonal antibodies.

2. CONJUGATE DILUENT:
1 x 20 ml. Contains thimerosal 0.02% and gentamicin 0.1% as preservatives.
Ready to use.

3. CALIBRATORS:
1 x 1.5 ml of calibrator A
1 x 0.5 ml of calibrators B-F.
Calibrated against WHO 2nd IRP 80/558. The calibrator values are approximately 0, 0.50, 1.5, 5.0, 15, 30 mIU/l. Exact levels are given on a separate sheet for each specific lot. Contain thimerosal 0.02% and gentamicin 0.1% as preservatives.
Ready to use.

4. BIOTIN CONJUGATE:
1 x 12 ml of anti-TSH monoclonal antibodies labelled with biotin. Contains thimerosal 0.02% and gentamicin 0.1% as preservatives. Ready to use.

5. STREPTAVIDIN CONJUGATE:
1 x 60 µl of streptavidin labelled with peroxidase. Concentrate 300x. Dilute with conjugate diluent just before use. Contains thimerosal 0.02% and gentamicin 0.1% as preservatives.

6. WASHING SOLUTION:
1 x 50 ml. Concentrate 20x. Before use dilute to 1000 ml with distilled water. Occasionally crystals may appear at 2-8°C. They dissolve at room temperature or when the concentrate is diluted.

7. SUBSTRATE/CHROMOGEN:
1 x 12 ml of 3,3', 5,5'-Tetrametilbenzidina (TMB). Ready to use.
Warning: store protected from light.

8. STOPPING SOLUTION:
1 x 12 ml of 1 N HCl. Ready to use.
Warning: avoid contact with eyes and skin.

9.SEALS ADHESIVE SEALS:
To cover the microplate during the incubations.

10.BAG RESEALABLE BAG:
For storage of unused strips.

## Storage and stability

All components will remain stable through the expiration date shown on the label if stored at 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, non used microplate wells should be kept in the plastic bag tightly sealed, with the silicagel and stored between 2-8°C. Diluted washing solution is stable for one week at room temperature or 3-4 weeks if stored at 2-8°C. Store the substrate/chromogen protected from light.

## PROCEDURE

Previous operations
Allow all reagents to reach room temperature (20-25°C) before use.

WASHING SOLUTION: dilute 1/20 with distilled water and mix carefully before use. Diluted washing solution is stable for one week at room temperature or 3-4 weeks if stored at 2-8°C.

STREPTAVIDIN-HRP CONJUGATE: prepare the working dilution according to the number of strips needed. The working streptavidin is not stable. Prepare only the quantity needed for the run.

| Strips required | 3 | 6 | 12 |
|---|---|---|---|
| Streptavidin-HRP conjugate | 10 µl | 20 µl | 40 µl |
| Conjugate diluent | 3 ml | 6 ml | 12 ml |

## Assay procedure

1. Identify the wells to be used for each calibrator and sample.

2. Pipette 100 µl of biotin conjugate into the wells.

3. Pipette 50 µl of calibrators in duplicate and serum samples into respective wells. Cover the microplate with an adhesive seal and shake it for a few seconds to mix the contents of the wells.

4. Incubate for 60 minutes at room temperature (20-25°C).

5. Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 4 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

6. Pipette 100 µl of diluted streptavidin-HRP conjugate into the wells.

7. Cover the plate with an adhesive seal and incubate for 60 minutes at room temperature (20-25°C).

8. Wash the wells as in step 5.

9. At timed intervals add 100 µl of substrate/chromogen solution into each well.

10. Incubate for 20 minutes at room temperature (20-25°C).

11. Stop the reaction by adding 100 µl of stopping solution into each well at the same timed intervals as in step 9. Shake gently to mix the solutions.

12. Measure the absorbance at 450 - 620 nm using a microplate reader. Absorbance values are stable for 30 minutes protected from light.

## Quality control

It is recommended that internal controls be used in every assay.

Results
1. Calculate the mean absorbance for each duplicate.

2. Subtract the absorbance value of the calibrator zero from the mean absorbance values of calibrators, controls and samples

3. Draw the standard curve: plot in linear/linear coordinates the mean absorbance values of calibrators against their respective concentrations. Other qualified system for curve fitting may de used, eg., 4 parameter logistic (lin-lin).

4. TSH concentrations of controls and samples can be derived from their absorbances using the standard curve.

## Expected values

Serum samples of apparently healthy women and men were assayed using the bioelisa TSH assay, with the following results:

| Number of Samples | Total TSH mIU/l | |
|---|---|---|
| | Mean | Range |
| 146 | 1.8 | 0.32 - 5.2 |

Each laboratory should determine its own reference range.

# Performance characteristics

### Detection limit

On the basis of results of 34 replicate determinations of the zero calibrator, the minimum TSH concentration detectable by the present method is 0.029 mIU/l. The detection limit is defined as the value deviating by 2 SD from that of the zero calibrator.

### Precision

Intra- and inter-assay precisions were established by analysing six patient sera of different TSH concentrations. The results are shown in tables 1 and 2.

Table 1: Intra-assay precision

| Patient | Number of replicates | Mean mIU/l | SD | CV% |
|---|---|---|---|---|
| 1 | 16 | 0.141 | 0.0223 | 16.0 |
| 2 | 16 | 0.68 | 0.025 | 3.7 |
| 3 | 16 | 1.45 | 0.022 | 1.5 |
| 4 | 16 | 2.77 | 0.034 | 1.2 |
| 5 | 16 | 12.7 | 0.178 | 1. 4 |
| 6 | 16 | 19.4 | 0.638 | 3.3 |

Table 2: Inter-assay precision

| Patient | Number of replicates | Mean mIU/l | SD | CV% |
|---|---|---|---|---|
| 1 | 16 | 0.123 | 0.0186 | 15.1 |
| 2 | 16 | 0.69 | 0.041 | 5.9 |
| 3 | 16 | 1.35 | 0.046 | 3.4 |
| 4 | 16 | 2.61 | 0.055 | 2.1 |
| 5 | 16 | 11.7 | 0.411 | 3.5 |
| 6 | 16 | 20.1 | 0.894 | 4.4 |

### Recovery

A known amount of TSH was added to three patient sera and quantities recovered measured. The results are shown in table 3.

Table 3: Recovery

| Sample | Endogenous TSH mIU/l | Added TSH mIU/l | Expected TSH mIU/l | Observed TSH mIU/l | Recovery % |
|---|---|---|---|---|---|
| 1 | 0.84 | 1 | 1.84 | 1.76 | 96 |
| | | 5 | 5.84 | 5.77 | 99 |
| | | 10 | 10.8 | 10.6 | 98 |
| 2 | 4.1 | 1 | 5.1 | 4.9 | 96 |
| | | 5 | 9.1 | 8.2 | 90 |
| | | 10 | 14.1 | 12.4 | 88 |
| 3 | 10.8 | 1 | 11.8 | 11.4 | 97 |
| | | 5 | 15.8 | 14.5 | 92 |
| | | 10 | 20.8 | 17.8 | 86 |

**Linearity (dilution test)**

Eight patient samples were diluted with the zero calibrator to 1/2, 1/5 and 1/10. TSH values were assayed, and the results were corrected using dilution factors. Recovery results of the dilution tests are shown in table 4.

Table 4: Dilution of samples

| Sample | TSH mIU/l | Recovery % | | |
|---|---|---|---|---|
| | undiluted | 1/2 | 1/5 | 1/10 |
| 1 | 1.06 | 107 | 105 | 101 |
| 2 | 1.69 | 105 | 91 | 88 |
| 3 | 1.80 | 98 | 93 | 82 |
| 4 | 2.95 | 102 | 99 | 100 |
| 5 | 7.36 | 99 | 99 | 94 |
| 6 | 11.1 | 112 | 109 | 105 |
| 7 | 12.0 | 104 | 104 | 100 |
| 8 | 19.8 | 105 | 108 | 108 |

**Specificity**

The specificity of the TSH-test was determined by measuring the apparent TSH response caused by high levels of hLH, hFSH, hCG. The results of these cross-reaction tests are shown in table 5.

Table 5: Cross reactions

| Substance | Concentration | Apparent TSH value mIU/l |
|---|---|---|
| hLH: Scripps Laboratories Affinity purified Cat L0815 calibrated against WH0 1st IRP 68/40 | 25 IU/l<br>50 IU/l<br>100 IU/l<br>200 IU/l<br>500 IU/l | < 0.15<br>< 0.15<br>< 0.15<br>< 0.15<br>< 0.15 |
| hFSH: Scripps Laboratories, Affinity purified Cat F0615 calibrated against WH0 1st IS 83/575 | 50 IU/l<br>100 IU/l<br>500 IU/l<br>1000 IU/l<br>4000 IU/l | < 0.15<br>< 0.15<br>< 0.15<br>< 0.15<br>< 0.15 |
| hCG : Scripps Laboratories, Jodination grade Cat C0714 calibrated against WH0 3rd IS 75/537 | 1000 IU/l<br>5000 IU/l<br>10000 IU/l<br>25000 IU/l<br>50000 IU/l | < 0.15<br>< 0.15<br>< 0.15<br>< 0.15<br>< 0.15 |

**High-dose hook effect (pro-zone)**

To evaluate a possible hook effect (pro-zone), concentrations up to 50 000 mUI/l of TSH were assayed. No pro-zone was observed.

# bioelisa fT3

**ELISA test for the quantitative determination of free Triiodothyronine (fT3) in human serum.**

## Summary

Triiodothyronine, a thyroid hormone, circulates in blood almost completely bound (>99.5%) to carrier proteins. The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function, as the concentrations of the carrier proteins alters, the total triiodothyronine level changes so that the free triiodothyronine concentration remains constant. Thus, measurements of free triiodothyronine concentrations correlate more reliably with clinical status than total triiodothyronine levels. For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the free T3 concentration remains basically unchanged.
This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of free T3.

## Principle

Microplate wells are coated with anti-T3 antibodies. The test sample and calibrators are incubated simultaneously with HRP-T3 conjugate in the coated wells. Free T3 from the sample and HRP-T4 conjugate compete for a limited amount of anti-T3 antibodies on the solid phase. After a washing step, a colourless substrate/chromogen solution is added and allowed to incubate. This solution will develop a blue colour if the conjugate has bound to the coated well. The blue colour changes to yellow after blocking the reaction with hydrochloric acid. Colour intensity, which is measured spectrophotometrically is inversely proportional to the concentration of free T3 in the sample. By using several calibrators of known concentration, a dose response curve can be generated from which the concentration of an unknown sample can be ascertained.

## Components

1. MICROPLATE:
   12 x 8 wells coated with sheep antibodies anti-triiodothyronine.

2. CONJUGATE:
   1 x 12 ml of triiodothyronine -horseradish peroxidase (HRP) conjugate, in a protein matrix. Contains MIT (150 mg/l) and BND (150 mg/dl) as preservatives. Ready to use.

3. CALIBRATORS:
   6 x 1.0 ml of calibrators A-F.
   The calibrator concentrations are approximately of 0, 0.4, 1.2, 4.5, 8.0 and 18.0 pg/ml. Exact levels are given on a separate sheet for each specific lot. Contain MIT (150 mg/l), BND (150 mg/dl) and amphoteracin B (5 mg/ml) as preservatives. Ready to use.
   For conversion to SI units: 1pg/ml x 1.536 = pmol/l

4. WASHING SOLUTION:
   1 x 50 ml. Concentrate 20x. Before use dilute to 1000 ml with distilled water. Occasionally crystals may appear at 2 - 8 °C. They dissolve at room temperature or when the concentrate is diluted.

5. SUBSTRATE/CHROMOGEN:
1 x 12 ml of 3,3', 5,5'-Tetrametilbenzidina (TMB) in citrate buffer. Ready to use. Warning: store protected from light.

6. STOPPING SOLUTION:
1 x 12 ml of 1 N HCl. Ready to use.
Warning: avoid contact with eyes and skin.

7.SEALS ADHESIVE SEALS:
To cover the microplate during the incubations.

8.BAG RESEALABLE BAG:
For storage of unused strips.

## Storage and stability

All components will remain stable through the expiration date shown on the label if stored at 2-8°C. Once opened, the kit is stable for two months if stored at 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, non used microplate wells should be kept in the plastic bag tightly sealed, with the silicagel and stored between 2-8°C. Diluted washing solution is stable one week at room temperature or 2-3 weeks if stored at 2-8°C. Store the substrate/chromogen protected from light

# PROCEDURE

Previous operations
Allow all reagents to reach room temperature (20-25°C) before use.

WASHING SOLUTION: dilute 1/20 with distilled water and mix carefully before use. Diluted washing solution is stable for one week at room temperature or 2-3 weeks if stored at 2-8°C.

## Assay procedure

1.Identify the wells to be used for each calibrator and sample.

2.Pipette 50 µl of the appropriate calibrator or specimen, in duplicated, into the assigned wells.

3.Add 100 µl of conjugate solution to all wells. Cover the microplate with an adhesive seal and shake it for a few seconds to mix the contents of the wells.

4.Incubate for 60 minutes at room temperature (20-25°C).

5.Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 3 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

6.At timed intervals add 100 µl of substrate/chromogen solution into each well. Always add reagents in the same order to minimize reaction time differences between wells.

7.Incubate for 15 minutes at room temperature (20-25°C).

8.Stop the reaction by adding 100 µl of stopping solution into each well at the same timed intervals as in step 6. Shake gently to mix the solutions.

9.Measure the absorbance at 450 - 620 nm using a microplate reader. The results should be read within thirty (30) minutes of adding the stopping solution.

## Quality control

Each laboratory should assay internal controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80%, 50% and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## Results

1.Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

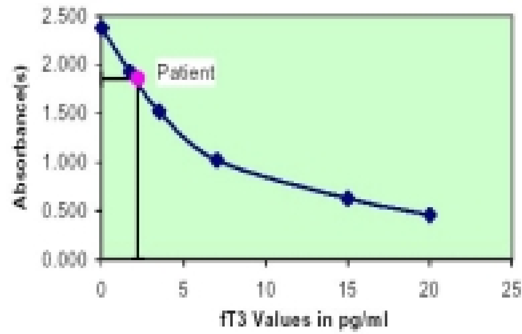
2.Plot the absorbance for each duplicate calibrator versus the corresponding fT3 concentration in pg/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).

3.Draw the best-fit curve through the plotted points.

4.To determine the concentration of fT3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml ) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.799 intersects the standard curve at 1.2 pg/ml fT3 concentration (see Figure 1)*.

| Well | Calibrators | Absorbance | | |
|---|---|---|---|---|
| 1 | 0.0 pg/ml | 2.639 | | |
| 2 | 0.0 pg/ml | 2.615 | | |
| 3 | 0.4 pg/ml | 2.330 | | |
| 4 | 0.4 pg/ml | 2.259 | | |
| 5 | 1.2 pg/ml | 1.835 | | |
| 6 | 1.2 pg/ml | 1.753 | | |
| 7 | 4.5 pg/ml | 0.939 | | |
| 8 | 4.5 pg/ml | 0.975 | | |
| 9 | 8.0 pg/ml | 0.537 | | |
| 10 | 8.0 pg/ml | 0.659 | | |
| 11 | 18.0 pg/ml | 0.301 | | |
| 12 | 18.0 pg/ml | 0.287 | | |
| | **Sample** | **Absorbance** | **Average** | **Concentration** |
| 13 | 1 | 1.876 | 1.799 | 1.2 pg/ml |
| 14 | 1 | 1.721 | | |

Figure 1




*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay.

## Assay validation

Absorbance of calibrator A (0 pg/ml): ≥ 1.2

## Expected values

A study of euthyroid adult population was undertaken to determine expected values for the bioelisa fT3 assay. Results are presented in table 1.

Table 1: Expected values for the free T3 (in pg/ml)

| | Adult (110 samples) | Pregnancy (75 samples) |
|---|---|---|
| Mean (x) | 2.8 | 3.0 |
| Standard deviation (SD) | 0.7 | 0.6 |
| Expected range (± 2 SD) | 1.4 - 4.2 | 1.8 - 4.2 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population from the area in which the laboratory is located.

## Performance characteristics

### Precision

The intra and inter assay precision of the fT3 were determined by analysis on three different levels of pool control sera. Results are presented in table 2 and table 3.

Table 2: Intra assay precision (values in pg/ml)

| Sample | N | Mean | SD | CV |
|---|---|---|---|---|
| Low | 20 | 1.37 | 0.16 | 11.9% |
| Normal | 20 | 4.21 | 0.17 | 4.1% |
| High | 20 | 7.1 | 0.17 | 2.4% |

Table 3: Inter assay precision* (values in pg/ml)

| Sample | N | Mean | SD | CV |
|---|---|---|---|---|
| Low | 10 | 1.4 | 0.15 | 10.7% |
| Normal | 10 | 4.4 | 0.23 | 5.2% |
| High | 10 | 7.0 | 0.30 | 4.2% |

*Measured in 10 experiments in duplicate, in a period of 10 days.

**Accuracy**
The bioelisa fT3 was compared with a coated tube radioimmunoassay analog method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (the values ranged from 0.1pg/ml - 14 pg/ml). The total number of such specimens was 85. The least square regression equation and the correlation coefficient were computed for this fT3 EIA in comparison with the reference method. The data obtained are displayed in table 4.

Table 4:

| Method | Mean (x) | Least square regression analysis | Correlation coefficient |
|---|---|---|---|
| bioelisa fT3 | 3.4 | y = 0.15 + 0.925 (x) | 0.995 |
| Reference | 3.5 | | |

**Sensitivity**
The triiodothyronine procedure has a sensitivity of 0.05 pg/ml. The sensitivity was ascertained by determining the variability (± 2SD) of the 0 pg/ml calibrator.

**Cross-reactivity**
The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of tracer.

| **Substance** | **Cross Reactivity** | **Concentration** |
|---|---|---|
| I-Triiodothyronine | 1.0000 | - |
| I-Thyroxine | < 0.0002 | 10µg/ml |
| Lodothyrosine | < 0.0001 | 10µg/ml |
| Diiodothyrosine | < 0.0001 | 10µg/ml |
| Diiodothyronine | < 0.0001 | 10µg/ml |
| Phenylbutazone | < 0.0001 | 10µg/ml |
| Sodium Salicylate | < 0.0001 | 10µg/ml |

# bioelisa fT4

**ELISA test for the quantitative determination of free Thyroxine (fT4) in human serum.**

## Summary

Thyroxine, the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is believed to be responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function, as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate more reliably with clinical status than total thyroxine levels. For example, the increase in total thyroxine levels associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the free thyroxine concentration remains in the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration. The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result. Again, the free thyroxine concentration typically uncovers the patient's actual clinical status.
This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of free T4.

## Principle

Microplate wells are coated with anti-T4 antibodies. The test sample and calibrators are incubated simultaneously with HRP-T4 conjugate in the coated wells. Free T4 from the sample and HRP-T4 conjugate compete for a limited amount of anti-T4 antibodies on the solid phase. After a washing step, a colourless substrate/chromogen solution is added and allowed to incubate. This solution will develop a blue colour if the conjugate has bound to the coated well. The blue colour changes to yellow after blocking the reaction with hydrochloric acid. Colour intensity, which is measured spectrophotometrically is inversely proportional to the concentration of free T4 in the sample. By using several calibrators of known concentration, a dose response curve can be generated from which the concentration of an unknown sample can be ascertained.

## Components

1. MICROPLATE:
   12 x 8 wells coated with sheep antibodies anti-thyroxine.

2. CONJUGATE:
   1 x 12 ml of thyroxine-horseradish peroxidase (HRP) conjugate, in a protein matrix. Contains MIT (150 mg/l) and BND (150 mg/dl) as preservatives. Ready to use.

3.CALIBRATORS:
   6 x 1.0 ml of calibrators A-F.
   The calibrator concentrations are approximately of 0, 0.3, 0.95, 2.1, 3.6 and 7.0 ng/dl. Exact levels are given on a separate sheet for each specific lot. Contain MIT (150 mg/l), BND (150 mg/dl) and amphoteracin B (5 mg/ml) as preservatives. Ready to use.

For conversion to SI units: 1ng/dl x 12.9 = pmol/l.

4. WASHING SOLUTION:
   1 x 50 ml. Concentrate 20x. Before use dilute to 1000 ml with distilled water. Occasionally crystals may appear at 2 - 8 °C. They dissolve at room temperature or when the concentrate is diluted.

5. SUBSTRATE/CHROMOGEN:
   1 x 12 ml of 3,3', 5,5'-Tetrametilbenzidina (TMB) in citrate buffer. Ready to use.
   Warning: store protected from light.

6. STOPPING SOLUTION:
   1 x 12 ml of 1 N HCl. Ready to use.
   Warning: avoid contact with eyes and skin.

7. SEALS ADHESIVE SEALS:
   To cover the microplate during the incubations.

8. BAG RESEALABLE BAG:
   For storage of unused strips.

## Storage and stability

All components will remain stable through the expiration date shown on the label if stored at 2-8°C. Once opened, the kit is stable for two months if stored at 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, non used microplate wells should be kept in the plastic bag tightly sealed, with the silicagel and stored between 2-8°C. Diluted washing solution is stable one week at room temperature or 2-3 weeks if stored at 2-8°C. Store the substrate/chromogen protected from light

**PROCEDURE (See summary of protocol in the last page)**
**Previous operations**
Allow all reagents to reach room temperature (20-25°C) before use.

WASHING SOLUTION: dilute 1/20 with distilled water and mix carefully before use. Diluted washing solution is stable for one week at room temperature or 2-3 weeks if stored at 2-8°C.

**Assay procedure**
1. Identify the wells to be used for each calibrator and sample.

2. Pipette 50 µl of the appropriate calibrator or specimen, in duplicated, into the assigned wells.

3. Add 100 µl of conjugate solution to all wells. Cover the microplate with an adhesive seal and shake it for a few seconds to mix the contents of the wells.

4. Incubate for 60 minutes at room temperature (20-25°C).

5. Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 3 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

6. At timed intervals add 100 µl of substrate/chromogen solution into each well. Always add reagents in the same order to minimize reaction time differences between wells.

7. Incubate for 15 minutes at room temperature (20-25°C).

8. Stop the reaction by adding 100 µl of stopping solution into each well at the same timed intervals as in step 6. Shake gently to mix the solutions.

9. Measure the absorbance at 450 - 620 nm using a microplate reader. The results should be read within thirty (30) minutes of adding the stopping solution.

## Quality control

Each laboratory should assay internal controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80%, 50% and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## Results

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate calibrator versus the corresponding fT4 concentration in ng/dl on linear graph paper (do not average the duplicates of the calibrators before plotting).

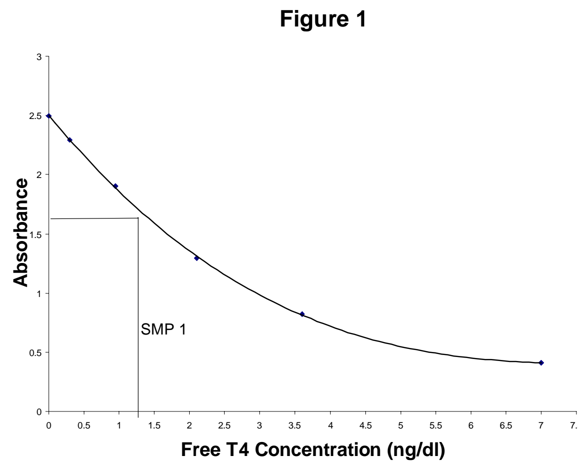
3. Draw the best-fit curve through the plotted points.

4. To determine the concentration of fT4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/dl ) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.730 intersects the standard curve at 1.3 ng/dl fT4 concentration (see Figure 1)*.

Example 1

| Well | Calibrators | Absorbance | | |
|---|---|---|---|---|
| 1 | 0.0 ng/dl | 2.462 | | |
| 2 | 0.0 ng/dl | 2.531 | | |
| 3 | 0.3 ng/dl | 2.330 | | |
| 4 | 0.3 ng/dl | 2.255 | | |
| 5 | 0.95 ng/dl | 1.915 | | |
| 6 | 0.95 ng/dl | 1.892 | | |
| 7 | 2.1 ng/dl | 1.328 | | |
| 8 | 2.1 ng/dl | 1.262 | | |
| 9 | 3.6 ng/dl | 0.834 | | |
| 10 | 3.6 ng/dl | 0.804 | | |
| 11 | 7.0 ng/dl | 0.399 | | |
| 12 | 7.0 ng/dl | 0.421 | | |
| | **Sample** | **Absorbance** | **Average** | **Concentration** |
| 13 | 1 | 1.783 | 1.730 | 1.3 ng/dl |
| 14 | 1 | 1.676 | | |

Figure 1




*The data presented in Example 1 and Figure 1 are for illustration only and should not be used instead of a standard curve prepared with each assay.

# Assay validation

Absorbance range of calibrator A (0 ng/dl): 1.500 - 2.700

# Expected values

A study of euthyroid adult population was undertaken to determine expected values for the **bioelisa fT4** assay. Results are presented in table 1.

Table 1: Expected values for the free T4 (in ng/dl)

| | Adult (110 samples) | Pregnancy (75 samples) |
|---|---|---|
| Mean (x) | 1.4 | 1.5 |
| Standard deviation (SD) | 0.6 | 0.7 |
| Expected range (± 2 SD) | 0.8 - 2.0 | 0.8 - 2.2 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population from the area in which the laboratory is located.

# Performance characteristics

## Precision

The intra and inter assay precision of the fT4 were determined by analysis on three different levels of pool control sera. Results are presented in table 2 and table 3.

Table 2: Intra assay precision (values in ng/dl)

| Sample | N | Mean | SD | CV |
|---|---|---|---|---|
| Low | 16 | 0.3 | 0.03 | 9.8% |
| Normal | 16 | 1.4 | 0.06 | 4.5% |
| High | 16 | 3.6 | 0.22 | 6.2% |

Table 3: Inter assay precision * (values in ng/dl)

| Sample | N | Mean | SD | CV |
|---|---|---|---|---|
| Low | 10 | 0.34 | 0.04 | 11.5% |
| Normal | 10 | 1.35 | 0.07 | 3.7% |
| High | 10 | 3.69 | 0.25 | 4.2% |

*Measured in 10 experiments in duplicate, in a period of 10 days.

**Accuracy**

The method was compared with a coated tube radioimmunoassay analog method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (the values ranged from 0.1ng/dl - 8ng/dl). The total number of such specimens was 85. The data obtained are displayed in table 4.

Table 4

| Method | Mean (x) | Least square regression analysis | Correlation coefficient |
|---|---|---|---|
| bioelisa fT4 | 1.5 | y = 0.10 + 0.952(x) | 0.978 |
| Reference | 1.4 | | |

**Sensitivity**

The test has a sensitivity of 0.05 ng/dl. The sensitivity was ascertained by determining the variability (± 2 SD) of the 0 ng/dl calibrator.

**Cross-reactivity**

The cross-reactivity of the thyroxine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyroxine needed to displace the same amount of conjugate.

| Substance | Cross Reactivity | Concentration |
|---|---|---|
| l -Thyroxine | 1.0000 | - |
| d-Thyroxine | 0.9800 | 10 ng/dl |
| d-Triiodothyronine | 0.0150 | 100 ng/dl |
| l -Triiodothyronine | 0.0300 | 100 ng/dl |
| Iodothyrosine | 0.0001 | 100 µg/ml |
| Diiodothyrosine | 0.0001 | 100 µg/ml |
| Diiodothyronine | 0.0001 | 100 µg/ml |

# bioelisa anti-Tg

**ELISA test for the quantitative determination of Thyroglobulin (Tg) auto-antibodies in human serum.**

## Summary

Antibodies to thyroglobulin have been shown to be characteristically present in patients with thyroiditis and primary thyrotoxicosis. This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Measurements of antibodies to Tg have been done, in the past, by Passive Haemagglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detection of subclinical levels of antibodies to Tg.

## Principle

In this method, calibrators and diluted patient specimens are added to microplate wells coated with streptavidin. Then biotinylated thyroglobulin (Tg) is added and the reactants are mixed. Reaction between the autoantibodies to Tg and the biotinylated Tg will form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin. After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate/chromogen to produce a blue colour. The blue colour changes to yellow after blocking the reaction with hydrochloric acid. Colour intensity, which is measured spectrophotometrically is proportional to the concentration of anti-Tg in the sample. By using several calibrators of known concentration, a dose response curve can be generated from which the concentration of an unknown sample can be ascertained.

## Components

1. MICROPLATE:
   12 x 8 wells coated with streptavidin.

2. SAMPLE DILUENT:
   1 x 20 ml of sample diluent containing buffer salts and a dye. Concentrate 10x. Before use dilute to 200 ml with distilled water.

3. CALIBRATORS:
   6 x 1.0 ml of calibrators A-F.
   Anti-Tg concentrations of calibrators are 0, 50, 125, 500, 1000 and 2000 IU/ml. Contain MIT (150 mg/l), BND (150 mg/dl) and amphoteracin B (5 mg/ml) as preservatives. The calibrators, human serum based, were adjusted using a reference preparation, which was assayed against the Medical Research Council (MRC) Standard A 65/93 for anti-thyroglobulin activity. Ready to use.

4. BIOTIN CONJUGATE:
   1 x 11 ml of biotinylated thyroglobulin in a buffering matrix. Contains MIT (150 mg/l), BND (150 mg/dl) and amphoteracin B (5 mg/ml) as preservatives. Ready to use.

5. ENZYME CONJUGATE:
   1 x 11 ml of anti-human IgG-horseradish peroxidase (HRP) conjugate in a buffering matrix. Contains MIT (150 mg/l) and BND (150 mg/dl) as preservatives. Ready to use.

6. WASHING SOLUTION:
   1 x 50 ml. Concentrate 20x. Before use dilute to 1000 ml with distilled water. Occasionally crystals may appear at 2-8°C. They dissolve at room temperature or when the concentrate is diluted.

7. SUBSTRATE/CHROMOGEN:
   1 x 12 ml of 3,3', 5,5'-Tetrametilbenzidina (TMB) in citrate buffer. Ready to use. Warning: store protected from light.

8. STOPPING SOLUTION:
   1 x 12 ml of 1 N HCl. Ready to use.
   Warning: avoid contact with eyes and skin.

9. SEALS ADHESIVE SEALS:
   To cover the microplate during the incubations.

10. BAG RESEALABLE BAG:
    For storage of unused strips.

## Storage and stability

All components will remain stable through the expiration date shown on the label if stored at 2-8°C. Opened reagents are stable for 2 months when stored at 2-8°C. The bag containing the microplate should be brought to room temperature before opening to avoid condensation in the wells. Once opened the bag, non used microplate wells should be kept in the plastic bag tightly sealed, with the silicagel and stored between 2-8°C. Diluted washing solution is stable for one week at room temperature or 2-3 weeks if stored at 2-8°C. Store the substrate/chromogen protected from light.

## PROCEDURE (See summary of protocol in the last page)

**Previous operations**

Allow all reagents to reach room temperature (20-25°C) before use.

WASHING SOLUTION: dilute 1/20 with distilled water and mix carefully before use. Diluted washing solution is stable for one week at room temperature or 2-3 weeks if stored at 2-8°C.

SAMPLE DILUENT*:* dilute to 200 ml in a suitable container with distilled or deionised water. Once diluted it is stable for sixty (60) days when stored at 2-8°C.

SAMPLE DILUTION (1/100)*:* Dispense 10 µl of each patient specimen into 1 ml of sample diluent. Cover and vortex or mix thoroughly by inversion. May be stored at 2-8°C for up to forty-eight (48) hours.

## Assay procedure

1. Identify the wells to be used for each calibrator and sample to be assayed in duplicate.

2. Pipette 50 µl of calibrators and diluted serum samples in duplicate into the assigned wells.

3. Add 100µl of Biotin conjugate. Cover the microplate with an adhesive seal and shake it for a few seconds to mix the contents of the wells.

4. Incubate for 60 minutes at room temperature (20-25°C).

5. Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 4 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle.

After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

6. Pipette 100 µl of HRP conjugate into the wells. Always add reagents in the same order to minimize reaction time differences between wells.

7. Cover the plate with an adhesive seal and incubate for 30 minutes at room temperature (20-25°C).

8. Wash the wells as in step 5.

9. At timed intervals add 100 µl of substrate/chromogen solution into each well.

10. Incubate for 15 minutes at room temperature (20-25°C).

11. Stop the reaction by adding 100 µl of stopping solution into each well at the same timed intervals as in step 9. Shake gently to mix the solutions.

12. Measure the absorbance at 450 - 620 nm using a microplate reader. Absorbance values are stable for 30 minutes protected from light.

**Note:** For re-assaying specimens with concentrations greater than 2000 IU/ml, dilute the sample an additional 1/10 or 1/50 using the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

## Quality control

Each laboratory should assay and establish reference intervals for clinically relevant controls to monitor assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80%, 50% and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## Results

A reference curve is used to ascertain the concentration of anti-Tg in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-Tg activity in IU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

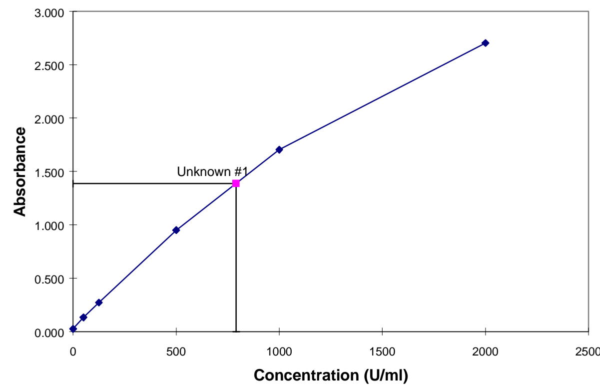
3. Draw the best-fit curve through the plotted points.

4. To determine the level of anti-Tg activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.387 (intersects the dose response curve) at 790 IU/ml anti-Tg concentration (see Figure 1).*

Example 1

| Well | Calibrators | Absorbance | | |
|---|---|---|---|---|
| 1 | 0 IU/ml | 0.022 | | |
| 2 | 0 IU/ml | 0.028 | | |
| 3 | 50 IU/ml | 0.135 | | |
| 4 | 50 IU/ml | 0.131 | | |
| 5 | 125 IU/ml | 0.280 | | |
| 6 | 125 IU/ml | 0.261 | | |
| 7 | 500 IU/ml | 0.962 | | |
| 8 | 500 IU/ml | 0.936 | | |
| 9 | 1000 IU/ml | 1.709 | | |
| 10 | 1000 IU/ml | 1.698 | | |
| 11 | 2000 IU/ml | 2.739 | | |
| 12 | 2000 IU/ml | 2.667 | | |
| | **Sample** | **Absorbance** | **Average** | **Concentration** |
| 13 | 1 | 1.390 | 1.387 | 790 IU/ml |
| 14 | 1 | 1.383 | | |

Figure 1




*The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a standard curve prepared with each assay.

# Assay validation

Maximum absorbance (calibrator 2000 IU/ml) = 1.500 – 3.500
Absorbance (calibrator 0 IU/ml) < 0.150
The ratio between the mean absorbance of each calibrator and preceding must be > 1.2

## Expected values

A study of normal population was undertaken to determine expected values for this anti-Tg assay. The number (n), mean (x) and standard deviation are given in table 1. Values in excess of 125 IU/ml are considered positive for the presence of anti-Tg auto-antibodies.

Table 1: Expected values for anti-Tg (IU/ml)

| | |
|---|---|
| Number (n) | 100 |
| Mean (x) | 74.3 |
| Standard deviation (SD) | 25.2 |
| Upper 95% (+ 2 SD) level | 124.7 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## Performance characteristics

### Precision

The intra and inter assay precision of the anti-Tg were determined by analysis on three different levels of pool control sera. Results are presented in table 2 and table 3.

Table 2: Intra assay precision (IU/ml)

| Sample | Number of replicates | Mean | SD | CV% |
|---|---|---|---|---|
| Pool 1 | 20 | 65.5 | 3.25 | 5.0% |
| Pool 2 | 20 | 385.5 | 15.5 | 4.0% |
| Pool 3 | 20 | 1554.4 | 55.4 | 3.6% |

Table 3: Inter assay precision* (IU/ml)

| Sample | Number of replicates | Mean | SD | CV% |
|---|---|---|---|---|
| Pool 1 | 10 | 66.8 | 3.6 | 5.3% |
| Pool 2 | 10 | 374.2 | 18.5 | 4.9% |
| Pool 3 | 10 | 1625.5 | 65.2 | 4.0% |

*Measured in 10 experiments in duplicate, in a period of 10 days.

### Accuracy

This Anti-Tg assay was compared with a reference microplate anti-Tg Elisa. Biological specimens from normal and disease states populations were used. The disease states included Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 82. The least square regression equation and the correlation coefficient were computed for the anti-Tg ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

Table 4

<table>
<tr><th>Method</th><th>Mean (x)</th><th>Least square regression analysis</th><th>Correlation coefficient</th></tr>
<tr><td>bioelisa anti-Tg</td><td>415.6</td><td rowspan="2">y = 9.79 + 0.969 (x)</td><td rowspan="2">0.995</td></tr>
<tr><td>Reference</td><td>419.2</td></tr>
</table>

Only slight amounts of bias between the anti-Tg ELISA system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

### Sensitivity

The test has a sensitivity of 5 IU/ml.

### Specificity

Interferences from ANA, DNA, thyroid peroxidase (TPO) and rheumatoid antibodies were found to be insignificant in the assay system.

# bioelisa anti-TPO

**ELISA test for quantitative determination of Thyroid Peroxidase (TPO) auto-antibodies in human serum.**


## Summary

Antibodies to thyroid peroxidase have been shown to be characteristically present from patients with Hashimoto thyroiditis (95%), idiopathic myxedema (90%) and Graves Disease (80%)[1]. In fact, 72% of patients positive for anti-TPO exhibit some degree of thyroid dysfunction.[2] This has lead to the clinical measurement becoming a valuable tool in the diagnosis of thyroid dysfunction. Measurements of antibodies to TPO have been done, in the past, by Passive Haemagglutination (PHA). PHA tests do not have the sensitivity of enzyme immunoassay and are limited by subjective interpretation. This procedure, with the enhanced sensitivity of EIA, permits the detectability of subclinical levels of antibodies to TPO.


## Principle

In this method, calibrators and diluted patient specimens are added to microplate wells coated with streptavidin. Then biotinylated Thyroid Peroxidase Antigen (TPO) is added and the reactants are mixed. Reaction between the autoantibodies to TPO and the biotinylated TPO will form an immune complex, which is deposited to the surface of streptavidin coated wells through the high affinity reaction of biotin and streptavidin. After the completion of the required incubation period, aspiration or decantation separates the reactants that are not attached to the wells. An enzyme anti-human IgG conjugate is then added to permit quantitation of reaction through interacting with human IgG of the immune complex. After washing, the enzyme activity is determined by reaction with substrate/chromogen to produce a blue colour. The blue colour changes to yellow after blocking the reaction with hydrochloric acid. Colour intensity, which is measured spectrophotometrically is proportional to the concentration of anti-TPO in the sample. By using several calibrators of known concentration, a dose response curve can be generated from which the concentration of an unknown sample can be ascertained.

## Components

1. MCPL MICROPLATE:
   12 x 8 wells coated with streptavidin.

2. SAMPLE DILUENT:
   1 x 20 ml of sample diluent containing buffer salts and a dye. Concentrate 10x. Before use dilute to 200 ml with distilled water.

3. CALIBRATORS:
   6 x 1.0 ml of calibrators A-F.
   Anti-TPO concentrations of the calibrators are 0, 25, 50, 100, 250 and 500 IU/ml. Contain MIT (150 mg/l), BND (150 mg/dl) and amphoteracin B (5 mg/ml) as preservatives. The calibrators, human serum based, were adjusted using a reference preparation, which was assayed against the Medical Research Council (MRC) Standard 66/287 for thyroid microsome. Ready to use.

4. BIOTIN CONJUGATE:
   1 x 11 ml of biotinylated thyroid peroxidase in a buffering matrix. Contains MIT (150 mg/l), BND (150 mg/dl) and amphoteracin B (5 mg/ml) as preservatives. Ready to use.

5. CONJUGATE:
   1 x 11 ml of anti-human IgG-horseradish peroxidase (HRP) conjugate in a buffering matrix. Contains MIT (150 mg/l) and BND (150 mg/dl) as preservatives. Ready to use.

6. WASHING SOLUTION:
   1 x 50 ml. Concentrate 20x. Before use dilute to 1000 ml with distilled water. Occasionally crystals may appear at 2-8°C. They dissolve at room temperature or when the concentrate is diluted.

7. SUBSTRATE/CHROMOGEN:
   1 x 12 ml of 3,3', 5,5'-Tetrametilbenzidina (TMB) in citrate buffer. Ready to use. Warning: store protected from light.

8. STOPPING SOLUTION:
   1 x 12 ml of 1 N HCl. Ready to use.
   Warning: avoid contact with eyes and skin.

9. ADHESIVE SEALS:
   To cover the microplate during the incubations.

10. RESEALABLE BAG:
   For storage of unused strips.

# PROCEDURE

**Previous operations**

Allow all reagents to reach room temperature (20-25°C) before use.

WASHING SOLUTION: dilute 1/20 with distilled water and mix carefully before use. Diluted washing solution is stable for one week at room temperature or 2-3 weeks if stored at 2-8°C.

SAMPLE DILUENT*:* dilute to 200 ml in a suitable container with distilled or deionised water. Once diluted it is stable for sixty (60) days when stored at 2-8°C.

SAMPLE DILUTION (1/100)*:* Dispense 10 µl of each patient specimen into 1 ml of sample diluent. Cover and vortex or mix thoroughly by inversion. May be stored at 2-8°C for up to forty-eight (48) hours.

## Assay procedure

1. Identify the wells to be used for each calibrator and sample to be assayed in duplicate.

2. Pipette 25 µl of calibrators and diluted serum samples in duplicate into the assigned wells.

3. Add 100µl of TPO-Biotin conjugate. Cover the microplate with an adhesive seal and shake it for a few seconds to mix the contents of the wells.

4. Incubate for 60 minutes at room temperature (20-25°C).

5. Remove and discard the adhesive seal. Aspirate the content of the wells and fill them completely (approximately 350 µl) with the diluted washing solution. Repeat the process of aspiration and washing 4 more times. Ensure that each column of wells soaks for at least 15 seconds before the next aspiration cycle. After the last washing blot the microplate on absorbent tissue to remove any excess liquid from the wells.

6. Pipette 100 µl of HRP conjugate into the wells. Always add reagents in the same order to minimize reaction time differences between wells.

7. Cover the plate with an adhesive seal and incubate for 30 minutes at room temperature (20-25°C).

8. Wash the wells as in step 5.

9. At timed intervals add 100 µl of substrate/chromogen solution into each well.

10. Incubate for 15 minutes at room temperature (20-25°C).

11. Stop the reaction by adding 100 µl of stopping solution into each well at the same timed intervals as in step 9. Shake gently to mix the solutions.

12. Measure the absorbance at 450 - 620 nm using a microplate reader. Absorbance values are stable for 30 minutes protected from light.

**Note:** For re-assaying specimens with concentrations greater than 500 IU/ml, dilute the sample an additional 1/10 or 1/50 using the original diluted material. Multiply by the dilution factor to obtain the concentration of the specimen.

## Quality control

Each laboratory should assay and establish reference intervals for clinically relevant controls to monitor assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80%, 50% and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

## Results

A reference curve is used to ascertain the concentration of anti-TPO in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.

2. Plot the absorbance for each duplicate serum reference versus the corresponding anti-TPO activity in IU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).

3. Draw the best-fit curve through the plotted points.

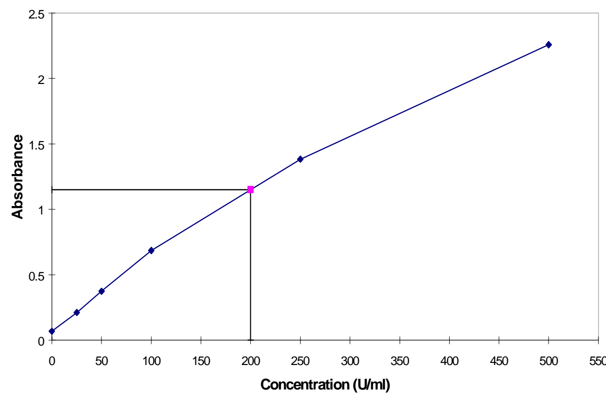
4. To determine the level of anti-TPO activity for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in IU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.150 (intersects the dose response curve) at 200 IU/ml anti-TPO concentration (see Figure 1).*

Example 1

| Well | Calibrators | Absorbance |
|---|---|---|
| 1 | 0 IU/ml | 0.068 |
| 2 | 0 IU/ml | 0.069 |
| 3 | 25 IU/ml | 0.209 |
| 4 | 25 IU/ml | 0.215 |
| 5 | 50 IU/ml | 0.380 |
| 6 | 50 IU/ml | 0.367 |
| 7 | 100 IU/ml | 0.691 |
| 8 | 100 IU/ml | 0.680 |
| 9 | 250 IU/ml | 1.400 |
| 10 | 250 IU/ml | 1.366 |
| 11 | 500 IU/ml | 2.312 |
| 12 | 500 IU/ml | 2.203 |

| | Sample | Absorbance | Average | Concentration |
|---|---|---|---|---|
| 13 | 1 | 1.125 | 1.150 | 200 IU/ml |
| 14 | 1 | 1.175 | | |

Figure 1




The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a standard curve prepared with each assay.

# Assay validation

Maximum absorbance (calibrator 500 IU/ml) = 1.500 – 3.500

Absorbance (calibrator 0 IU/ml) < 0.100

The ratio between the mean absorbance of each calibrator and preceding must be > 1.2

## Expected values

A study of normal population was undertaken to determine expected values for this anti-TPO assay. The number (n), mean (x) and standard deviation are given in table 1. Values in excess of 40 IU/ml are considered positive for the presence of anti-TPO auto-antibodies.

Table 1: Expected values for anti-TPO (IU/ml)

| | |
|---|---|
| Number (n) | 100 |
| Mean (x) | 17.6 |
| Standard deviation (SD) | 10.8 |
| Upper 95% (+ 2 SD) level | 39.2 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## Performance characteristics

### Precision

The intra and inter assay precision of the anti-TPO were determined by analysis on three different levels of pool control sera. Results are presented in table 2 and table 3.

Table 2: Intra assay precision (IU/ml)

| Sample | Number of replicates | Mean | SD | CV% |
|---|---|---|---|---|
| Pool 1 | 20 | 25.5 | 1.5 | 5.7% |
| Pool 2 | 20 | 120.5 | 4.6 | 3.8% |
| Pool 3 | 20 | 352.4 | 14.8 | 4.2% |

Table 3: Inter assay precision* (IU/ml)

| Sample | Number of replicates | Mean | SD | CV% |
|---|---|---|---|---|
| Pool 1 | 10 | 26.5 | 1.8 | 6.8% |
| Pool 2 | 10 | 118.5 | 5.3 | 4.5% |
| Pool 3 | 10 | 365.4 | 22.5 | 6.2% |

*Measured in 10 experiments in duplicate, in a period of 10 days.

### Accuracy

This Anti-TPO assay was compared with a reference microplate anti-TPO Elisa. Biological specimens from normal and disease states populations were used. The disease states included; Hashimoto's thyroiditis, Graves Disease, thyroid nodules as well as thyroid carcinoma. The total number of such specimens was 82. The least square regression equation and the correlation coefficient were computed for the bioelisa anti-TPO in comparison with the reference method. The data obtained is displayed in Table 4.

Table 4

| Method | Mean (x) | Least square regression analysis | Correlation coefficient |
|---|---|---|---|
| bioelisa anti-TPO | 122.9 | y = 1.02 (x) - 5.1 | 0.989 |
| Reference | 127.0 | | |

Only slight amounts of bias between the anti-Tg ELISA system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

**Sensitivity**

The test has a sensitivity of 1.5 IU/ml.

**Specificity**

Interferences from ANA, DNA, thyroglobulin (Tg) and rheumatoid antibodies were found to be insignificant in the assay system.

## bioelisa thyroid kits Selling points

| |
|---|
| ✓ Accurate diagnosis of the thyroid function |
| ✓ Convenient Breakable wells |
| ✓ Highly Sensitive, Specific and Precise |
| ✓ Easy to automate: High volume throughput |
| ✓ Most of reagents ready to use |
| ✓ Correlates to RI A value |
| ✓ Accurately differentiates Euthyroidism, Hypothyroidism and Hyperthyroidism |
| ✓ CE Marked |

## Evaluations with WHO standard and biorad controls

### bioelisa TSH

bioelisa TSH was evaluated against the WHO standard and the biorad Lyphocheck Immunoassay Plus Control Levels 1, 2 and 3

| WHO standard | Nominal concentration IU/ml | reported concentration IU/ml |
|---|---|---|
| neat | 20 | 17.9 |
| dilution 1/8 | 2.5 | 2.0 |

| biorad Control | Acceptable range IU/ml | reported concentration IU/ml |
|---|---|---|
| Level 1 | 0.12 – 0.55 | 0.155 |
| Level 2 | 3.6 – 9.5 | 4.22 |
| Level 3 | 18.9 - 54 | 25.45 |

### bioelisa fT3

bioelisa fT3 was evaluated against the biorad Lyphocheck Immunoassay Plus Control Levels 1, 2 and 3

| biorad Control | Acceptable range pg/ml | reported concentration pg/ml |
|---|---|---|
| Level 1 | 0.7 -3.9 | 1.98 |
| Level 2 | 4.8 – 7.2 | 5.17 |
| Level 3 | 13.2 – 19.8 | 12.3 |

### bioelisa fT4

bioelisa fT4 was evaluated against the biorad Lyphocheck Immunoassay Plus Control Levels 1, 2 and 3

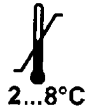
| biorad Control | Acceptable range ng/ml | reported concentration ng/ml |
|---|---|---|
| Level 1 | 0.3 – 0.63 | 0.6 |
| Level 2 | 4.8 – 7.2 | 5.17 |
| Level 3 | 13.2 – 19.8 | 12.3 |

# bioelisa TSH

**1x96 TESTS** REF **3000-1145**

| | |
|---|---|
| 1 | MCPL |
| 1 x 12 ml | BIOT CONJ |
| 1 x 60 µl | STRE CONJ 300x |
| 1 x 20 ml | DIL CONJ |
| 1 x 50 ml | WASH SOLN 20x |
| 1 x 12 ml | SUBS TMB |
| 1 x 1.5 ml | CAL A |
| 5 x 0.5 ml | CAL B C D E F |
| 1 x 12 ml | HCl 1N |
| 1 | BAG |
| | SEALS |

IVD




CE

# bioelisa TSH

**1x96 TESTS** REF **3000-1145**

ELISA test for the quantitative determination of Thyroid-stimulating hormone (TSH) in human serum or plasma / test de ELISA para la determinación cuantitativa de la hormona estimulante del tiroides (TSH) en suero o plasma humano / test ELISA per la determinazione quantitativa dell'ormone stimolante la tiroide (TSH) in siero o plasma umano.

LOT **I-3504**
⌛ **2005-06-26**

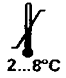
biokit — bioelisa TSH — CAL E — 0.5 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU — 2...8°C

biokit — bioelisa TSH — CAL F — 0.5 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU — 2...8°C

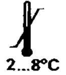
biokit — bioelisa TSH — HCl 1N — 12 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU — 2...8°C

Fecha 05.11.04
V° B°

biokit — bioelisa TSH — MCPL — IVD — LOT I-3504 — ⌛ 2005-06-26

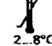



biokit — bioelisa TSH — WASH SOLN 20x — 50 ml — IVD — LOT I-3504 — ⌛ 2005-06-26

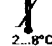



biokit — bioelisa TSH — BIOT CONJ — 12 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU

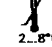
biokit — bioelisa TSH — STRE CONJ 300x — 60 µl — IVD — LOT I-3504 — ⌛ 2005-06-26




biokit — bioelisa TSH — DIL CONJ — 20 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU



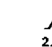

biokit — bioelisa TSH — SUBS TMB — 12 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU



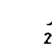

biokit — bioelisa TSH — CAL A — 1.5 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU

biokit — bioelisa TSH — CAL B — 0.5 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU

biokit — bioelisa TSH — CAL C — 0.5 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU




biokit — bioelisa TSH — CAL D — 0.5 ml — IVD — LOT I-3504 — ⌛ 2005-06-26 — RTU



## bioelisa fT3

1x96 TESTS REF 3000-1146

| | |
|---|---|
| 1 | MCPL |
| 1 x 12 ml | CONJ |
| 1 x 50 ml | WASH SOLN 20x |
| 1 x 12 ml | SUBS TMB |
| 6 x 1.0 ml | CAL A B C D E F |
| 1 x 12 ml | HCl 1N |
| 1 | BAG |
| | SEALS |

IVD

2...8°C

CE

## bioelisa fT3

1x96 TESTS REF 3000-1146

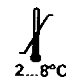
ELISA test for the quantitative determination of free Triiodothyronine (fT3) in human serum / test de ELISA para la determinación cuantitativa de Triyodotironina libre (fT3) en suero humano / test ELISA per la determinazione quantitativa della Triiodotironina libera (fT3) in siero umano.

LOT K-2004
2005-06-26

biokit bioelisa fT3 HCl 1N 12 ml IVD LOT K-2004 2005-06-26 RTU 2...8°C

GARANTÍA DE CALIDAD
Fecha 05-11-04
V.º B.º

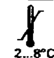
biokit bioelisa fT3 MCPL IVD LOT K-2004 2005-06-26


2...8°C

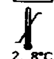
biokit bioelisa fT3 WASH SOLN 20x 50 ml IVD LOT K-2004 2005-06-26

2...8°C

biokit bioelisa fT3 CONJ 12 ml IVD LOT K-2004 2005-06-26 RTU


2...8°C

biokit bioelisa fT3 SUBS TMB 12 ml IVD LOT K-2004 2005-06-26 RTU


2...8°C

biokit bioelisa fT3 CAL A 1.0 ml IVD LOT K-2004 2005-06-26 RTU

2...8°C

biokit bioelisa fT3 CAL B 1.0 ml IVD LOT K-2004 2005-06-26 RTU

2...8°C

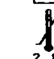
biokit bioelisa fT3 CAL C 1.0 ml IVD LOT K-2004 2005-06-26 RTU

2...8°C

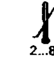
biokit bioelisa fT3 CAL D 1.0 ml IVD LOT K-2004 2005-06-26 RTU

2...8°C

biokit bioelisa fT3 CAL E 1.0 ml IVD LOT K-2004 2005-06-26 RTU


2...8°C

biokit bioelisa fT3 CAL F 1.0 ml IVD LOT K-2004 2005-06-26 RTU

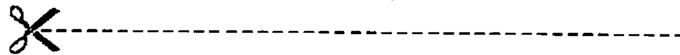

2...8°C

# bioelisa fT4

1x96 TESTS REF 3000-1147

| | |
|---|---|
| 1 | MCPL |
| 1 x 12 ml | CONJ |
| 1 x 50 ml | WASH SOLN 20x |
| 1 x 12 ml | SUBS TMB |
| 6 x 1.0 ml | CAL A B C D E F |
| 1 x 12 ml | HCl 1N |
| 1 | BAG |
| | SEALS |

IVD 2...8°C CE



# bioelisa fT4

1x96 TESTS REF 3000-1147

ELISA test for the quantitative determination of free Thyroxine (fT4) in human serum / test de ELISA para la determinación cuantitativa de Tiroxina libre (fT4) en suero humano / test ELISA per la determinazione quantitativa della Tiroxina libera (fT4) in siero umano.

LOT K-2104
2005-06-26

biokit bioelisa fT4
HCl 1N 12 ml IVD
LOT K-2104
2005-06-26 RTU
2...8°C

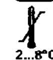
GARANTÍA DE CALIDAD
Fecha 05.11.04
Vº B.º

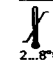
biokit bioelisa fT4
MCPL IVD
LOT K-2104
2005-06-26

2...8°C

biokit bioelisa fT4
WASH SOLN 20x 50 ml IVD
LOT K-2104
2005-06-26

2...8°C

biokit bioelisa fT4
CONJ 12 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

biokit bioelisa fT4
SUBS TMB 12 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

biokit bioelisa fT4
CAL A 1.0 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

biokit bioelisa fT4
CAL B 1.0 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

biokit bioelisa fT4
CAL C 1.0 ml IVD
LOT K-2104
2005-06-26 RTU

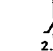
2...8°C

biokit bioelisa fT4
CAL D 1.0 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

biokit bioelisa fT4
CAL E 1.0 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

biokit bioelisa fT4
CAL F 1.0 ml IVD
LOT K-2104
2005-06-26 RTU

2...8°C

# bioelisa anti-TPO

**1x96 TESTS** REF 3000-1151

| | |
|---|---|
| 1 | MCPL |
| 1 x 11 ml | CONJ |
| 1 x 11 ml | BIOT CONJ |
| 1 x 20 ml | DIL SAMP 10x |
| 1 x 50 ml | WASH SOLN 20x |
| 1 x 12 ml | SUBS TMB |
| 6 x 1.0 ml | CAL A B C D E F |
| 1 x 12 ml | HCl 1N |
| 1 | BAG |
| | SEALS |

IVD

2...8°C

# bioelisa anti-TPO

**1x96 TESTS** REF 3000-1151

ELISA test for the quantitative determination of Thyroid Peroxidase (TPO) autoantibodies in human serum / test de ELISA para la determinación cuantitativa de auto-anticuerpos contra la Tireo-peroxidasa (TPO) en suero humano / test ELISA per la determinazione quantitativa degli autoanticorpi anti-Tireo perossidasi (TPO) in siero umano.

8 436003 074694

LOT K-2604
2005-06-26

GARANTÍA DE CALIDAD
Fecha 04-11-04
V.º B.º

biokit bioelisa anti-TPO
MCPL
IVD
LOT K-2604
2005-06-26

2...8°C

biokit bioelisa anti-TPO
WASH SOLN 20x
50 ml
IVD
LOT K-2604
2005-06-26

2...8°C

biokit bioelisa anti-TPO — CONJ — 11 ml — IVD — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — BIOT CONJ — 11 ml — IVD — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — DIL SAMP 10x — 20 ml — IVD — LOT K-2604 — 2005-06-26 — 2...8°C

biokit bioelisa anti-TPO — SUBS TMB — 12 ml — IVD — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — CAL A — 1.0 ml — IVD — IU/ml 0 — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — CAL B — 1.0 ml — IVD — IU/ml 25 — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — CAL C — 1.0 ml — IVD — IU/ml 50 — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — CAL D — 1.0 ml — IVD — IU/ml 100 — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — CAL E — 1.0 ml — IVD — IU/ml 250 — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

biokit bioelisa anti-TPO — CAL F — 1.0 ml — IVD — IU/ml 500 — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

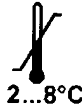
biokit bioelisa anti-TPO — HCl 1N — 12 ml — IVD — LOT K-2604 — 2005-06-26 — RTU — 2...8°C

# bioelisa anti-Tg

**1x96 TESTS** REF 3000-1150

| | |
|---|---|
| 1 | MCPL |
| 1 x 11 ml | CONJ |
| 1 x 11 ml | BIOT CONJ |
| 1 x 20 ml | DIL SAMP 10x |
| 1 x 50 ml | WASH SOLN 20x |
| 1 x 12 ml | SUBS TMB |
| 6 x 1.0 ml | CAL A B C D E F |
| 1 x 12 ml | HCl 1N |
| 1 | BAG |
| | SEALS |

IVD



# bioelisa anti-Tg

**1x96 TESTS** REF 3000-1150

ELISA test for the quantitative determination of Thyroglobulin (Tg) auto-antibodies in human serum / test de ELISA para la determinación cuantitativa de auto-anticuerpos contra la Tiroglobulina (Tg) en suero humano / test ELISA per la determinazione quantitativa degli autoanticorpi anti-Tireoglobulina (Tg) in siero umano.

LOT K-2504

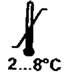
2005-06-26

biokit — bioelisa anti-Tg — CAL F — 1.0 ml — IVD — IU/ml 2000 — LOT K-2504 — 2005-06-26 — RTU — 2...8°C

biokit — bioelisa anti-Tg — HCl 1N — 12 ml — IVD — LOT K-2504 — 2005-06-26 — RTU — 2...8°C

CALIDAD
Fecha 04-11-04
V.º B.º

biokit — bioelisa anti-Tg — MCPL — IVD — LOT K-2504 — 2005-06-26




biokit — bioelisa anti-Tg — WASH SOLN 20x — 50 ml — IVD — LOT K-2504 — 2005-06-26

biokit — bioelisa anti-Tg — CONJ — 11 ml — IVD — LOT K-2504 — 2005-06-26 — RTU

biokit — bioelisa anti-Tg — BIOT CONJ — 11 ml — IVD — LOT K-2504 — 2005-06-26 — RTU

biokit — bioelisa anti-Tg — DIL SAMP 10x — 20 ml — IVD — LOT K-2504 — 2005-06-26

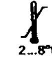
biokit — bioelisa anti-Tg — SUBS TMB — 12 ml — IVD — LOT K-2504 — 2005-06-26 — RTU

biokit — bioelisa anti-Tg — CAL A — 1.0 ml — IVD — IU/ml 0 — LOT K-2504 — 2005-06-26 — RTU

biokit — bioelisa anti-Tg — CAL B — 1.0 ml — IVD — IU/ml 50 — LOT K-2504 — 2005-06-26 — RTU

biokit — bioelisa anti-Tg — CAL C — 1.0 ml — IVD — IU/ml 125 — LOT K-2504 — 2005-06-26 — RTU

biokit — bioelisa anti-Tg — CAL D — 1.0 ml — IVD — IU/ml 500 — LOT K-2504 — 2005-06-26 — RTU




biokit — bioelisa anti-Tg — CAL E — 1.0 ml — IVD — IU/ml 1000 — LOT K-2504 — 2005-06-26 — RTU