# DRI® Thyroxine (T4) Assay

Thermo SCIENTIFIC

**IVD** **For In Vitro Diagnostic Use**

**Rx Only**

**REF** 0454 (100 mL Kit)
10013070 (500 mL Kit)

## Intended Use
This homogeneous thyroxine enzyme immunoassay is intended to be used for quantitative determination of total thyroxine in human serum or plasma.

## Summary and Explanation of the Test
Thyroxine (T4) is synthesized within the follicles of the thyroid gland and released into the blood circulation through a complex feedback system[1]. The thyroid gland is regulated by the thyroid stimulating hormone (TSH) which is produced and secreted by the pituitary gland. The production and secretion of TSH by the pituitary is through the stimulation by the thyroid releasing hormone (TRH) which is released by the hypothalamus.

Most thyroxine in blood circulation is predominantly bound to thyroxine binding globulin (TBG) and to a lesser extent to thyroxine binding albumin and prealbumin[2,3]. Only less than 1% of thyroxine remains unbound as free T4 in blood. Elevated total thyroxine levels have been associated with hyperthyroidism, a condition with an excess amount of circulating thyroid hormone and decreased total thyroxine levels have been associated with hypothyroidism, a condition with insufficient levels of thyroxine concentration. Primary malfunction of the thyroid gland or any diseases affecting the thyroid-pituitary-hypothalamus system may result in the abnormal thyroxine concentration in blood. Measurement of total thyroxine concentration (free plus protein-bound) has been one of the most widely used method for evaluation of an individual's thyroid status[4].

The DRI® Thyroxine Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents. The assay uses 8-anilino-1-naphthalene sulfonic acid (ANS) to dissociate thyroxine from the plasma binding proteins. The dissociated thyroxine in the sample is allowed to compete with an enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled thyroxine for a fixed amount of anti-thyroxine specific antibody binding sites in the solution. In the absence of thyroxine from the sample, the G6PDH labeled thyroxine is bound by the specific antibody and the enzyme activity is inhibited. This phenomenon creates a relationship between thyroxine concentration in the sample and the enzyme activity. The enzyme G6PDH activity is determined spectrophotometrically at 340 nm by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH.

## Materials Provided
**Antibody/Substrate Reagent.** Contains monoclonal anti-thyroxine antibody, 8-anilino-naphthalene sulfonic acid (ANS), glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as preservative.
**Enzyme Conjugate Reagent.** Contains thyroxine labeled with glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as preservative.

***Calibrators (Sold separately)***
**Thyroxine Calibrator Kit (Cat # 0476):** Contains 2 mL each of negative, 2 µg/dL, 4 µg/dL, 8 µg,dL, 12 µg/dL and 20 µg/dL thyroxine calibrators in human serum with sodium azide as a preservative.

## ⚠ Precautions and Warning
This test is for in vitro diagnostic use only. The reagents are harmful if swallowed.

**DANGER:** DRI Thyroxine (T4) Assay contains ≤0.1% bovine serum albumin (BSA).
H317 - May cause allergic skin reaction.
H334 - May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Avoid breathing mist or vapor. Contaminated work clothing should not be allowed out of the workplace. Wear protective gloves/eye protection/face protection. In case of inadequate ventilation wear respiratory protection. If on skin: Wash with plenty of soap and water. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. If skin irritation or rash occurs: Get medical advice/attention. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. Wash contaminated clothing before reuse. Dispose of contents/container to location in accordance with local/regional/national/international regulations.

Reagents used in the assay components contain ≤ 0.09% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Get immediate medical attention for eyes, or if ingested. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up. Clean exposed metal surfaces with 10% sodium hydroxide.

Do not use the reagents beyond their expiration dates.

Thyroxine is light sensitive. Store all reagents containing thyroxine (enzyme conjugate, calibrators, controls or patient samples) in a way as to minimize exposure to direct sunlight.

In case of damaged packaging on arrival, contact your technical support representative.

## Reagent Preparation and Storage
The reagents are ready for use. No reagent preparation is required. All assay components, when stored properly at 2°C to 8°C are stable until the expiration date indicated on the label.

## Specimen Collection and Handling
Either serum or plasma can be used with the assay. Anticoagulants, such as heparin, citrates, oxalates and EDTA, were found not to interfere with the assay. Fresh serum specimen is preferred. If the sample can not be analyzed immediately, it may be stored refrigerated for up to one week or frozen for up to one month. Repeated freezing and thawing of the sample should be avoided. An effort should be made to keep samples free of gross debris. It is recommended that highly turbid specimens be centrifuged before analysis.

**Handle all serum or plasma specimens as if they were potentially infectious.**[5]

## Instruments
Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reaction accurately can be used to perform this homogenous enzyme immunoassay.

## Assay Procedure
Chemistry analyzers with the indicated specifications are suitable for performing this homogeneous enzyme immunoassay. Refer to the specific application instructions for each assay and specific parameters used for each analyzer before performing the assay.

## Quality Control and Calibration
Good laboratory practice suggests the use of control specimens to validate the calibration and to ensure proper assay performance. Various multi-level commercial controls are available for this purpose. Ensure that control results are within the established range. Recalibrate the system when new reagents are used or when the control values are outside the established range. All quality control requirements should be performed in conformance with local, state and/or federal regulations or accreditation requirements.

## Results and Data Interpretation
The sample thyroxine concentration results are calculated automatically by the clinical chemistry analyzer. No additional data manipulation is required.

Samples quantitating greater than 20 µg/dL can be reported as > 20 µg/dL or diluted using the negative calibrator. Diluted sample value is obtained by multiplying the result by the dilution factor.

Various factors can affect the relationship between the serum or plasma thyroxine concentration and clinical response. These include patient's age, sex and state of health, specific drug therapy, non-thyroidal illness, pregnancy, use of estrogen or contraceptives and genetic increase or decrease of thyroid binding globulin (TBG) concentration. With the above mentioned circumstances, total thyroxine should only be used as a preliminary screening procedure.[1] Accurate diagnosis of thyroid status should be supplemented with other diagnostic tests such as Free T4 Index (FTI), TSH, T3, TRH, etc., and physician's clinical evaluation.

## Expected Values
The range of T4 concentrations of apparently healthy individuals has been determined to be 4.5 to 12 µg/dL. Since the "normal" ranges can be affected by age, gender, diet, geographic location and other factors, each laboratory should establish its own expected values for this procedure.

## Limitations
Sensitivity, defined as the lowest concentration that can be differentiated from the negative serum with 95% confidence, is 0.7 µg/dL. This assay is optimized for the determination of thyroxine in serum or plasma only and not for whole blood thyroxine determination. In rare situations, patients may have autoantibody that will interfere with the assay and result in low test results.

## Typical Performance Characteristics
The following typical performance data were generated on the Hitachi 717 clinical chemistry analyzer:

***Precision***
The within-run and total run precision were evaluated with three levels of T4 serum controls. Following a modified CLSI precision protocol, samples were tested in replicates of 6 per run, twice a day for 5 days, with total N = 60.

| Level | | Within-run | | Total-run | |
|---|---|---|---|---|---|
| n=60 | Mean (µg/dL) | SD (µg/dL) | CV% | SD (µg/dL) | CV% |
| 1 | 4.1 | 0.14 | 3.4 | 0.28 | 6.9 |
| 2 | 11.0 | 0.41 | 3.7 | 0.81 | 7.4 |
| 3 | 16.2 | 0.62 | 3.8 | 1.05 | 6.5 |

***Sensitivity***
Sensitivity, defined as the lowest concentration that can be differentiated from the negative serum with 95% confidence, is 0.7 µg/dL.

***Accuracy***
108 clinical samples with T4 concentration range from 1.3 µg/dL to 87.1 µg/dL were assayed with DRI Thyroxine EIA assay and a commercially available T4 assay. A correlation with a regression equation of DRI (y) = 1.02 (x) - 0.63 and a correlation coefficient (r) of 0.993 was obtained.

***Specificity***
Compounds with chemical structure similar to that of thyroxine and certain concurrently used compounds were tested for possible cross reactivity in the thyroxine assay. The % cross reactivity was determined as the percent of equivalent T4 concentration observed when the tested concentration of the cross reactant was added to a T4 negative serum.

| Compound | Conc. Tested (µg/dL) | % Cross Reactivity |
|---|---|---|
| Triiodothyronine (T3) | 10 | 3.2* |
| Triiodothyroacetic Acid | 10 | 0.5* |
| Tetraiodothyroacetic Acid | 10,000 | 25.3* |
| 3,5-Diiodothyronine | 10,000 | 0.0 |
| 3,5-Diiodotyrosine | 10,000 | 0.0 |
| Iodotyrosine | 10,000 | 0.0 |
| Methimazole | 10,000 | 0.0 |
| Phenylbutazone | 10,000 | 0.0 |
| Phenytoin | 10,000 | 0.0 |
| Propylthiouracil | 10,000 | 0.0 |
| Tyrosine | 10,000 | 0.0 |
| Acetaminophen | 100,000 | 0.0 |
| Acetylsalicylic Acid | 100,000 | 0.0 |

* The tested concentrations greatly exceed the normal serum concentrations of these compounds. Therefore the cross reactivity is not clinically significant.

Hemolyzed (up to 800 mg/dL hemoglobin), lipemic (up to 1000 mg/dL triglycerides or 400 mg/dL cholesterol) and icteric (up to 30 mg/dL bilirubin) samples have no clinically significant interference on the assay.

**Bibliography**


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4. Penney M, O'Sullivan J. Total or Free Thyroxine as a Primary Test of Thyroid Function. Clin. Chem., 33, 170 (1987).
5. Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories". 1988.


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Microgenics Corporation
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EC REP
B·R·A·H·M·S GmbH
Neuendorfstrasse 25
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