DRI® T-Uptake Assay

Thermo SCIENTIFIC

IVD **For In Vitro Diagnostic Use Only**

Rx Only

REF 0723 (34 mL,100 mL Kit)

Intended Use
This homogeneous enzyme immunoassay is intended to be used for quantitative determination of unsaturated binding sites on the thyroid binding proteins in human serum or plasma.

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

Most thyroxine in blood circulation is predominantly bound to the thyroxine binding globulin (TBG) and to a lesser extent to the thyroxine binding prealbumin and albumin. Less than 1% of thyroxine remains unbound as free T4 in blood. Free, or unbound fractions of the thyroid hormone are thought to be responsible for the biologic activity. Patients with a hyperthyroid condition have increased levels of T4 and thus, a decreased number of unsaturated binding sites on the thyroid binding proteins. Patients with a hypothyroid condition have decreased levels of T4 and consequently, an increased number of unsaturated binding sites on the thyroid binding proteins.[2,3]

Several physiological conditions such as pregnancy, estrogen therapy, chronic illness or congenital disorders can alter the concentration of TBG in serum.[4-6] These variations can lead to abnormal T4 concentrations in individuals with no thyroid disease. Therefore, use of the total T4 concentration alone may not provide an accurate indication of an individual's thyroid status.

The product of the T-Uptake value and the total T4 concentration is called the Free Thyroxine Index (FTI).[7] FTI provides a clinically useful and accurate estimate of the circulating free thyroxine. Therefore, the determination of unsaturated thyroid-binding capacity along with the total T4 provides a better indication of thyroid status than a total T4 determination alone.

The DRI T-Uptake Assay is a homogeneous enzyme immunoassay in a ready to use liquid format. A mixture of enzyme glucose-6-phosphate dehydrogenase (G6PDH) conjugated thyroxine and a known amount of exogenous thyroxine are allowed to bind to the thyroxine binding proteins in the serum sample. In the case of a hypothyroid sample with an increased level of unsaturated thyroid binding sites, the exogenous T4 will bind to the unsaturated binding sites leaving the G6PDH-T4 conjugate free. Upon addition of an anti-thyroxine antibody, the G6PDH-T4 conjugate is bound by the antibody and the enzyme activity is inhibited. Conversely, a hyperthyroid sample with decreased level of unsaturated thyroxine binding sites will leave most exogenous T4 unbound. Upon addition of anti-T4 antibody, the unbound exogenous T4 will inhibit the anti-T4 binding to G6PDH-T4 conjugate and produce a high G6PDH enzyme activity. This phenomenon creates a direct relationship between the unsaturated thyroxine binding sites concentration (T-Uptake) in sample and the enzyme activity. The enzyme 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, glucose-6-phosphate (G6P), and nicotinamide adenine dinucleotide (NAD) in Tris buffer with sodium azide as preservative.
Enzyme Conjugate Reagent. Contains thyroxine and thyroxine-labeled glucose-6-phosphate dehydrogenase (G6PDH) in Tris buffer with sodium azide as preservative.

Calibrators and Controls (Sold separately)
DRI T-Uptake Calibrator Kit (Cat # 0738): Contains 2 mL each of 15%, 20%, 30%, 40% and 50% human serum-based T-Uptake Calibrators with sodium azide as a preservative. These calibrators are required for assay calibration

T-Uptake Controls: Available from various commercial sources.

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

DANGER: DRI T-Uptake 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: Ger 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 in light sensitive. Store all reagents containing thyroxine (enzyme conjugate, calibrators, controls or patient samples) in a way as to mininmize exposure to direct sunlight.

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 and kept from contamimination (before or after bottle opening), 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.[8] A fresh serum specimen is preferred. If the sample cannot be analyzed immediately, it may be stored refrigerated at 2-8ºC for up to one week. Samples may be stored frozen at -20ºC for up to one month. Repeated freezing and thawing of the same 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 urine specimens as if they were potentially infectious.[9]

Instruments
Clinical chemistry analyzers capable of maintaining a constant temperature, pipetting samples, mixing reagents, measuring enzymatic rates at 340 nm and timing the reacting 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, available from Microgenics, a part of Thermo Fisher Scientific 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. Use all calibrators to generate the standard curve. Prepare a new standard curve when a new set of reagents is used. A typical calibration curve, generated with a Hitachi 717 analyzer is as follows:

Calibrator (%TU)	Rate
15	106
20	130
30	149
40	166
50	182

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 T-Uptake values can be calculated automatically by the clinical chemistry analyzer. No additional data manipulation is required.

Various physiological factors can alter the T-Uptake values or thyroxine binding globulin (TBG) concentrations. These factors include pregnancy, estrogen therapy, chronic illness or congenital disorders. The T-Uptake assay result should not be used alone as a means to determine hyperthyroidism or hypothyroidism. It should be used in conjunction with total T4 to determine the FTI for estimating the circulating free thyroxine concentrations. The FTI value should be calculated by the following equation:[7]

$$FTI = \frac{\text{(Total T4 µg/dL) x (\% T-Uptake)}}{28\%^*}$$

* 28% is the mean T-Uptake value established by Microgenics study. Each laboratory should use the T-Uptake value established with its own patient population for FTI calculation.

In rare instances, individuals having antibodies to thyroxine can depress the enzyme activity, which may lead to falsely lower results. Coupled with the above mentioned condition, accurate diagnosis of thyroid status should be supplemented with other diagnostic tests such as TSH, T3, TRH, etc., and the physician's clinical evaluation.

Expected Values

The mean normal T-Uptake in 150 apparently healthy individuals was established with DRI T-Uptake assay as 28% with a range of 23-36%. Since the mean or the expected T-Uptake ranges can be affected by age, sex, diet, geographical location and other factors, each laboratory should establish its own expected T-Uptake values for normal and/or abnormal thyroid status.

Limitations

The assay is optimized for use with serum or plasma only and not for whole blood application. In rare situations, patients may have autoantibodies that will interfere with the assay and result in low test results. The assay range is 15-50%. Any values below 15% should be reported as <15%. No sample dilution should be made.

Typical Performance Characteristics

The following performance data were generated with a Hitachi 717 analyzer:

Precision

The within-run and run-to-run precision were evaluated with three levels of T-Uptake. The run-to-run data was generated with duplicate determinations and collected over a two-week period.

Within-run (n=10)		Run-to-run (n=10)	
Mean (%TU)	**C.V (%)**	**Mean (%TU)**	**C.V (%)**
15.0	0.1%	15.0	0.5%
30.2	3.6%	29.6	2.7%
38.6	2.8%	38.7	3.3%

Accuracy

104 clinical samples were assayed with the DRI T-Uptake EIA Assay (y) and a commercially available EIA assay (x). The FTI results obtained from both assays indicated a good correlation with a regression equation of DRI (y) = 0.92 (x) + 0.69, a correlation coefficient (r) of 0.9 and sample FTI ranges of 3.79-16.97 (for DRI) and 4.20-16.95 (for commercial EIA assay).

Interfering Substances

Hemolyzed (up to 800 mg/dL hemoglobin), lipemic (up 400 mg/dL cholesterol) and icteric (up to 14 mg/dL bilirubin) samples have no clinically significant effect on the assay.

Bibliography

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Glossary:

http://www.thermofisher.com/symbols-glossary

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