Thyroxine (T4)
Biolis Liquid Reagent
(24 Tray)

Glenbio
Diagnostics & Research Laboratory Solutions

KIT SPECIFICATIONS:

Catalogue No.	*Quantity*	*Reagent*	*Storage*
GLC24086	R1: 2 x 25 mL	Thyroxine R1	2 – 8 °C
	R2: 2 x 8.5 mL	Thyroxine R2	

INTENDED USE:

In Vitro Diagnostic reagent pack used for the quantitative determination of total thyroxine in human serum or plasma on Biolis automated analysers.

SUMMARY AND EXPLANATION:

The Glenbio Thyroxine Assay is a homogeneous enzyme immunoassay using ready-to-use liquid reagents.
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].

PRINCIPLE OF THE TEST:

The Glenbio Thyroxine 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) labelled 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 labelled 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.

WARNINGS AND PRECAUTIONS:

For In Vitro Diagnostics Use Only – For Professional Use Only.
Carefully read instructions for use. Deviations from this procedure may alter the performance of the assay. In the case of serious damage to the bottle and/or cap, resulting in product leakage and/or contamination, do not use the reagent pack and contact your distributor.

Safety Precautions:
Material Safety Data Sheet available upon request. The reagents are harmful if swallowed.
DANGER: 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 vapour. 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. The reagents contain sodium azide (<0.1%) as a preservative. Avoid contact with skin and mucous membranes.

Handling Precautions:
- Take the necessary precautions required for handling all laboratory reagents.
- Do not use components past the expiry date stated on the Bottles.
- Do not use components for any purpose other than described in the "Intended Use" section.
- Do not interchange caps among components as contamination may occur and compromise test results. After measurements are taken, reagent bottles should be capped and kept at 2 - 8°C.
- Refer to local legal requirements for safe waste disposal.
- Reagents with different lot numbers should not be interchanged or mixed.
- Reagents are light sensitive, store in a place out of direct sunlight.

COMPONENT COMPOSITION:

	Contains:
Reagent 1	Monoclonal anti-thyroxine antibody, 8-anilino-naphthalene sulfonic acid (ANS), Glucose-6-phosphate (G6P), Nicotinamide adenine dinucleotide (NAD) , Tris buffer, Sodium azide (preservative)
Reagent 2	Thyroxine labelled with glucose-6-phosphate dehydrogenase (G6PDH), Tris buffer, Sodium azide (preservative)

REAGENT PREPARATION AND STABILITY:

Reagent 1 and 2 are ready for use.
If stored unopened and handled correctly, the components are stable until the expiry date stated on the label. Reagents from different lots must not be interchanged. Protect from light and avoid contamination.

TYPE OF SPECIMEN:

Serum or plasma (EDTA, heparin, oxalates and citrates were found not to interfere with this assay).

Handle all specimens as if they were potentially infectious.

If the test cannot be completed immediately, the sample may be placed in a tightly sealable container and stored refrigerated for up to one week or frozen for up to 4 weeks. Repeated freezing and thawing should be avoided. It is recommended to perform the assay with freshly collected samples. It is recommended that highly turbid specimens be centrifuged before analysis.

TEST PROCEDURE:

Materials required but not supplied:

Description	Catalogue No.
Thyroxine Calibrator Set	GL9729
General Laboratory Equipment	N/A
Biolis Analyser and Consumables	N/A

Assay procedure:
Refer to relevant user's manual for instructions on instrument start-up, loading components and samples, calibration, sample testing procedures, calculating and reporting results.

Calibration:
Using the recommended Calibrator, calibrate the assay:
- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Controls are out of range.

Quality Control:
All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program.
Controls should be assayed:
- Prior reporting patient results.
- Following any maintenance procedure.
- At intervals established by the Laboratory QC programme.

RESULTS:

Results are calculated automatically by the analyser.

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:

Range of T4 concentrations of apparently healthy individuals	4.5 to 12 µg/dL

It is recommended for each laboratory to establish its own reference ranges for local population as "normal" ranges can be affected by gender, age, diet, geographical location and other factors. Diagnosis should only be made after taking clinical symptoms and the results of other tests into consideration.

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.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Sensitivity:
0.7 µg/dL
(Defined as the lowest concentration that can be differentiated from the negative serum with 95% confidence.)

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.

Interference:
The following have no clinically significant interference on the assay:

Haemoglobin	Up to 800 mg/dL.
Bilirubin	Up to 30 mg/dL.
Triglycerides	Up to 1000 mg/dL.
Cholesterol	Up to 400 mg/dL.

Precision:

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

Accuracy:
Using 108 samples, a comparison, between this Thyroxine test (y) and another commercially available test (x), gave the following results:

y = 1.02x – 0.63	r = 0.993	Sample range: 1.3 to 87.1 µg/dL

BIBLIOGRAPHY:

1. Ingbar SH, Woeber KA. The Thyroid Gland. In: Text Book of Endocrinology. Williams RH. ed. Philadelphia, PA; WB Saunders Company, 95 (1974).
2. Robbins J. "Thyroxine-Binding Protein in Serum" In: Laborator Diagnosis of Endocrine Disease. Saunderman and Saunderman eds., St. Louis, MO; Warren H. Green, Inc., 221 (1971).
3. Larsen PR, et al. "Immunoassay of Thyroxine in Unextracted Human Serum", J. Clin. Endocrinol. Metal., 37, 177 (1973).
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.

SYMBOLS:

The following symbols are used in the labelling of Glenbio Ltd. systems:

IVD	In Vitro Diagnostics	REF	Catalogue No
LOT	Batch Code	CONT	Content
REAG	Reagent	Ab	Antibody
CAL	Calibrator	SUBS	Substrate
BUF	Buffer	CE	CE Mark - Device complies with the Directives 98/79/EC
	Storage temperature	→	Reconstitute with
	Expiry Date (Last day of the month)		Manufactured By
	Biological risk		Consult Instruction for Use

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* For Reagent Instrument Application Settings please contact: **applications@glenbio.com**

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