T4

KAPDB4240

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve - Belgium

LOT : 110225/1

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T4

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For the direct quantitative determination of Thyroxine by enzyme immunoassay in human serum.

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IN VITRO DIAGNOSTIC

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INTENDED USE

For the direct quantitative determination of Thyroxine by enzyme immunoassay in human serum.
For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in calibrators, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of T4 in the sample. A set of calibrators is used to plot a calibration curve from which the amount of T4 in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

Clinical Trends:

- The level of T4 in hypothyroid patients is decreased.
- The level of T4 in hyperthyroid patients is increased.
- In euthyroid individuals the level of T4 is within the normal range.

Thyroid binding globulin (TBG) levels have reportedly been elevated by the following conditions: increased estrogens from oral contraceptives, androgens, glucocorticords and pregnancy. Consequently, borderline T4 values should be viewed with caution when any of the above conditions are present.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
6. A calibration curve must be established for every run.
7. The control should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. The assay buffer is sensitive to light and should be stored in the original dark bottle away from direct sunlight.
12. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
13. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator and control.
14. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
15. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of T4 in human serum. The kit is not calibrated for the determination of T4 in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the calibrators and control has been tested and found to be non-reactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. However no test method can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered as potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 ml of serum is required per duplicate determination. Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SERUM PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µl
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED

Mouse Anti-T4 Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.
Contents: One 96 well (12x8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

AG	HRP	CONC

T4-Horseradish Peroxidase (HRP) Conjugate Concentrate – X25
Contents: T4-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 1 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:25 in assay buffer before use (eg. 80 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 800 µl of HRP in 20 ml of assay buffer. Discard any that is left over.

CAL	N

T4 Calibrators - Ready To Use. N = 0 to 4

Contents: Five vials containing T4 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of T4.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume
Calibrator 0	0 µg/dl	2.0 ml
Calibrator 1	1 µg/dl	0.5 ml
Calibrator 2	4 µg/dl	0.5 ml
Calibrator 3	12 µg/dl	0.5 ml
Calibrator 4	32 µg/dl	0.5 ml

Storage: Refrigerate at 2-8°C
Stability: 12 months in unopened vials or as indicated on label. Once opened, the calibrators should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

CONTROL **Control** - Ready To Use.

Contents: One vial containing T4 in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of T4. Refer to vial label for expected value and acceptable range.
Volume: 0.5 ml/vial
Storage: Refrigerate at 2-8 °C
Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

WASH SOLN CONC **Wash Buffer Concentrate - X10**

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.
Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

ASS BUF **Assay Buffer** - Ready To Use.

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Volume: 25 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

CHROM TMB **TMB Substrate** - Ready To Use.

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 ml/bottle
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

STOP SOLN **Stopping Solution** - Ready To Use.

Contents: One vial containing 1M sulfuric acid.
Volume: 6 ml/vial
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

ASSAY PROCEDURE
Specimen Pretreatment:
None.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the T4-HRP conjugate and wash buffer.
2. Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 20 µl of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 150 µl of the conjugate working solution into each well (We recommend using a multichannel pipette).
5. Incubate on a plate shaker (approximately 200 rpm) for 30 minutes at room temperature.
6. Wash the wells 3 times with 300 µl of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 150 µl of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 15-20 minutes at room temperature (or until calibrator 0 attains dark blue colour for desired OD).
9. Pipette 50 µl of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microwell plate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

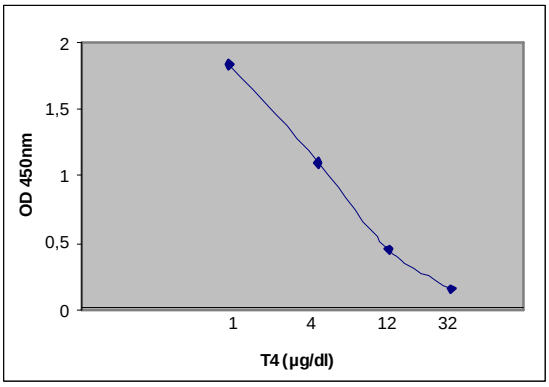
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibration curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the calibration curve.
5. If a sample reads more than 32 µg/dl then dilute it with calibrator 0 at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (µg/dl)
0	2.176	2.174	2.175	0
1	1.850	1.820	1.835	1
2	1.092	1.109	1.101	4
3	0.434	0.468	0.451	12
4	0.152	0.158	0.155	32
Unknown	0.635	0.634	0.635	8.3

TYPICAL CALIBRATION CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the calibration curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of the DIAsource Direct T4 ELISA kit is
0.6 µg/dl.

SPECIFICITY (CROSS REACTIVITY)

The following compounds were tested for cross-reactivity with the Direct T4 ELISA kit with T4 cross-reacting at 100%.

Compound	%Cross Reactivity
L-Thyroxine	100
D-Thyroxine	94
3,3',5'-Triiodo-L-Thyronine (Reverse T3)	86
3,3',5-Triiodo-L-Thyronine (T3)	3.3
3,3',5'-Triiodo-D-Thyronine	1.8
3,3',5'-Triiodothyropropionic acid	0.6

The following compounds were tested but cross-reacted at less than 0.04%: Acetylsalicylic acid, 3,5-Diiodo-L-Thyronine, 3,5-Diiodo-L-Tyrosine and 3-Iodo-L-Tyrosine.

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibration curve. The results (in µg/dl) are tabulated below:

Sample	Mean	SD	CV%
1	2.48	0.23	9.2
2	8.58	0.60	6.9
3	20.46	1.33	6.4

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µg/dl) are tabulated below:

Sample	Mean	SD	CV%
1	3.33	0.41	12.3
2	10.30	1.19	11.5
3	14.5	1.44	9.9

RECOVERY

Spiked samples were prepared by adding defined amounts of T4 to three patient serum samples. The results (in µg/dl) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1 Unspiked	2.03	-	-
+2.91	4.64	4.94	93.9
+7.38	9.28	9.41	98.6
+13.70	17.93	15.73	114.0
2 Unspiked	9.43	-	-
+2.91	13.17	12.32	106.9
+7.38	19.56	16.81	116.4
+13.70	25.81	23.13	111.6
3 Unspiked	24.03	-	-
+2.91	26.74	26.94	99.3
+7.38	30.65	31.41	97.6
+13.70	>32	37.73	-

LINEARITY

Two patient serum samples were diluted with calibrator 0. The results (in µg/dl) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	22.50	-	-
1:2	11.74	11.25	104.4
1:4	5.70	5.63	101.2
1:8	2.71	2.81	96.4
2	25.64	-	-
1:2	14.50	12.82	113.1
1:4	6.90	6.41	107.6
1:8	3.31	3.21	103.1

EXPECTED VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µg/dl)
Euthyroid	4-12
Hyperthyroid	>12
Hypothyroid	<4

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Revision date : 2011-02-25

	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
I V D	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor