Instruction for use

A SOLID-PHASE ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF FREE TRIIODOTHYRONINE IN HUMAN BLOOD SERUM OR PLASMA

1. INTENDED USE

A solid-phase enzyme immunoassay for the quantitative determination of free triiodothyronine in blood serum or plasma.

This kit is designed for measurement of free triiodothyronine in blood serum or plasma. For possibility of use with other sample types, please, refer to Application Notes (on request). The kit contains reagents sufficient for 96 determinations and allows to analyze 41 unknown samples in duplicates.

2. SUMMARY AND EXPLANATION

Thyroid hormones thyroxin (T4) and 3,5,3'-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. T4 and T3 in blood are found both in free and bound form – mostly, they are bound to thyroxin binding globulin (TBG). Only free forms of T3 and T4 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for T4.

The concentration of T3 is much less than that of T4 but its metabolic activity is about 3 times greater. About 80% of T3 is produced in peripheral tissues by deiodination of T4, and only 20% is secreted by thyroid gland. That is why in hypothyroid patients T3 level may for a long time remain on the lower limit of the normal range, because its loss may be compensated by enhanced conversion of T4 into T3.

Determination of T3 level is most useful in T3-hyperthyroidism because 5-10% of such patients do not show significant changes in T4 level while concentration of T3 is highly elevated.

Elevated T3 levels are seen in early thyroid hypofunction, after intake of estrogens, oral contraceptives, heroin, methadone, during pregnancy.

Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates.

3. PRINCIPLE OF THE TEST

This test is based on competition enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific rabbit polyclonal to T3-antibodies simultaneously with conjugated fT3-peroxidase. fT3 from the specimen competes with the conjugated fT3 for coating antibodies. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is inversely related to the quantity of the measured analyte in the specimen.

4. WARNINGS AND PRECAUTIONS

4.1. For professional use only.

4.2. This kit is intended for in vitro diagnostic use only.

4.3. INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

4.4. Avoid contact with stop solution containing 5,0% H_2SO4. It may cause skin irritation and burns.

4.5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.

4.6. Do not use the kit beyond the expiration date.

4.7. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.

4.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

4.9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

4.10. Do not mix reagents from different lots.

4.11. Replace caps on reagents immediately. Do not swap caps.

4.12. Do not pipette reagents by mouth.

4.13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.

4.14. Safety Data Sheet for this product is available upon request directly from XEMA Co., Ltd.

4.15. The Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

5. KIT COMPONENTS

5.1. Contents of the Kit

	Symbol	Description		Qty	Units	Colour code	Stability of opened/diluted components
1	SORB MTP	fT3 EIA strips, 8x12 wells	polystyrene microwells coated with rabbit polyclonal to T3	1	pcs		until exp. date
2	CAL 1–6	Calibrator set, 0.8 ml each. The set contains 6 calibrators: 0; 2.5; 5; 10; 20, 40 pmol/l	human free triiodothyronine diluted in a preselected human serum preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains bright blue dye	6	pcs	bright blue (C1 - colourless)	2 months
3	CONTROL	Control serum (0.8 ml)	dilution of preselected human serum, with high content of free triiodothyronine with preservative - 0,01% Bronidox L, 0,01% 2-Methyl-4-isothiazolin-3-one-hydrochloride, colourless	1	pcs	colourless	2 months
4	CONJ HRP	Conjugate, 5.2 ml	aqueous solution of T3 coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and blue dye	1	pcs	blue	until exp.date
5	SUBS TMB	Substrate solution, 11 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
6	BUF WASH 21X	Washing solution concentrate 21x, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate - until exp.date Diluted washing solution - 1 month at 2...+8 °C or 5 days at RT
7	STOP	Stop solution, 11 ml	5,0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp. date
8	N003	Plate sealing tape		2	pcs		N/A
9	K213I	Instruction fT3 EIA		1	pcs		N/A
10	K213Q	QC data sheet fT3 EIA		1	pcs		N/A

5.2. Equipment and material required but not provided
- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µl, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µl;
- Dry thermostat for 37 °C ±0.1 °C
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0.

5.3. Storage and stability of the Kit

Store the whole kit at +2…+8 °C upon receipt until the expiration date.

After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

6. SPECIMEN COLLECTION AND STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/thawing should be avoided.

7. TEST PROCEDURE

7.1. Reagent Preparation
- All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.
- All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
- Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

7.2. Procedural Note:

It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

See the example of calibration graphic in Quality Control data sheet.

7.3. Assay flowchart

See the example of calibration graphic in Quality Control data sheet.

7.4. Assay procedure

1	Put the desired number of microstrips into the frame; allocate 14 wells for the calibrators CAL 1–6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2	Pipet 50 µl of calibrators CAL 1–6, control samples CONTROL and unknown samples into the wells.
3	Dispense 50 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape (included into the kit).
4	Incubate 60 minutes at 37 °C.
5	Prepare washing solution by 21x dilution of washing solution concentrate (BUF WASH 21X) by distilled water. Wash the strips 5 times.
6	Dispense 100 µl of SUBS TMB into the wells
7	Incubate 10–20 minutes at +18…+25 °C
8	Dispense 100 µl of STOP into the wells.
9	Measure OD (optical density) at 450 nm.
10	Set photometer blank on air
11	Apply lin-log method for data reduction.

7.5. Handing notes
Calibrators and control sample(s) – only one freezing/thawing cycle is allowed.

8. QUALITY CONTROL

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

9. CALCULATION OF RESULTS

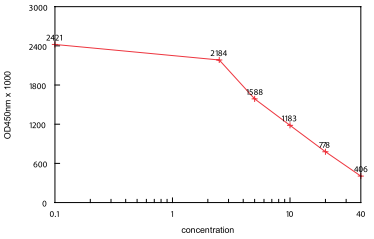
1. Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.

2. Plot a calibration curve on graph paper: OD versus free triiodothyronine concentration.

3. Determine the corresponding concentration of free triiodothyronine in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

4. Below is presented a typical example of a standard curve with the XEMA Co. Not for calculations!

Calibrators	Value	Absorbance Units (450 nm) x 1000
CAL 1	0 pmol/l	2421
CAL 2	2.5 pmol/l	2184
CAL 3	5 pmol/l	1588
CAL 4	10 pmol/l	1183
CAL 5	20 pmol/l	778
CAL 6	40 pmol/l	406



10. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for T4. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Sex, age	Units, pmol/l	
	Lower limit	Upper limit
Healthy donors	2.5	5.8

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical specificity / Cross reactivity

Analyte	Cross-reactivity, % wt/wt
L-T3	100
D-T3	100
L-Thyroxin	0.01
D-Thyroxin	0.04

11.2. Analytical sensitivity

Sensitivity of the assay was assessed as being 1 pmol/l.

11.3. Linearity

Linearity was checked by assaying dilution series of 5 samples with different free triiodothyronine concentrations. Linearity percentages obtained ranged within 90 to 110%.

11.4. Recovery

Recovery was estimated by assaying 5 mixed samples with known free triiodothyronine concentrations. The recovery percentages ranged from 90 to 110%.

12. LITERATURE

1. Physiology of thyroid hormones. IN: Division of Drugs and Toxicology, American Medical Association: Drug Evaluations Annual 1995. Amer Med Assn, Chicago, 1995, ch 47, pp 1039-1040.

2. Robins J & Rall JE. The Iodine -Containing Hormones. IN Hormones in Blood (2nd ed) 1: 383-490, Gray CH & Bacharach AL (eds) London Academic Press, 1987