 **WELDON BIOTECH**

***Inspiration for Life Sciences***

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| **Wel-*Lisa* Free  *Triiodothyronine* Hormone (FT3) ELISA** |

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**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

***SUMMARYAND EXPLANATION***

Thyroid hormones thyroxin (FT3) and 3,5,3’-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. FT3 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 FT3 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for FT3.

The concentration of T3 is much less than that of FT3 but its metabolic activity is about 3 times greater. About 80% of T3 is produced in peripheral tissues by deiodination of FT3, 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 FT3 into T3. Determination of T3 level is most useful in T3-hyperthyroidism because 5-10% of such patients do not show significant changes in FT3 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.Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates

***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.

***WARNINGS AND PRECAUTIONS***

1. For professional use only.
2. This kit is intended for in vitro diagnostic use only.
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. Avoid contact with stop solution containing 5,0% H2SO4. It may cause skin irritation and burns.
5. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.
6. Do not use the kit beyond the expiration date.
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.
8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
9. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.
10. Do not mix reagents from different lots.
11. Replace caps on reagents immediately. Do not swap caps.
12. Do not pipette reagents by mouth.
13. Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.
14. Safety Data Sheet for this product is available upon request directly from WEL-LISA Co., Ltd.
15. The Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

**KIT COMPONENTS**

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No.** | ***Symbol*** | ***Description*** | | ***Qty*** | ***Units*** | ***Colour code*** | ***Stability of opened/diluted components*** |
| 1 | SORB MTP | FT3 EIA strips, 8х12 wells | polystyrene microwells coated with murine monoclonal to FT3 | 1 | No. | -- | 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 | No. | red (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 | No. | colourless | 2 months |
| 4 | CONJ HRP | Conjugate, 14 ml | aqueous solution of T3 coupled with horseradish peroxidase diluted on phosphate buffered solution preservative - 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride and blue dye | 1 | No. | red | until exp.date |
| 5 | SUBS TMB | Substrate solution,14 ml | ready-to-usesingle-component tetramethylbenzidine (TMB) solution. | 1 | No. | colourless | until exp.date |
| 6 | BUF WASH 26X | Washing solution concentrate 26x, 22 ml | aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative | 1 | No. | colourless | Concentrate - until exp.date Diluted washing solution - 45 days at 2-8 °C or 15 days at RT |
| 7 | STOP | Stop solution, 14 ml | 5,0% vol/vol solution of sulphuric acid | 1 | No. | colourless | until exp.date |
| 8 | N003 | Plate sealing tape | - | 2 | No. | - | N/A |
| 9 | K2131 | Instruction fFT3 EIA | - | 1 | No. | - | N/A |
| 10 | K213Q | QC data sheet FT3 EIA | - | 1 | No. | - | N/A |

**EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED:**

1. Distilled or deionized water
2. Precision pipettes with variable volume.
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance

at 450nm

1. Absorbance paper or paper towel
2. Graph paper
3. Dry Thermo state for 37 ̊C ± 0.1 ̊C

***STORAGE AND STABILITY OF THE KIT***

*microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture*

***SPECIMEN COLLECTION AND STORAGE.***

*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.*

# TEST PROCEDURE

***Reagent Preparation***

*All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18 to +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.*

***Procedural Note:***

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

**ASSAY**

See the example of calibration graphic in Quality Control data sheet

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 25 µl of calibrators CAL 1 - 6, control samples CONTROL and unknown samples into the wells.

3 Dispense 100 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 26x dilution of washing solution concentrate (BUF WASH 26X) 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.

**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.

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| --- | --- | --- |
| **Calibrators** | **Value** | **AbsorbanceUnits**  **(450 nm) x 1000** |
| **CAL 1** | 0 pmol/l | 2401 |
| **CAL 2** | 2.5 pmol/l | 2164 |
| **CAL 3** | 5.0 pmol/l | 1515 |
| **CAL 4** | 10 pmol/l | 1145 |
| **CAL 5** | 20 pmol/l | 775 |
| **CAL 6** | 40 pmol/l | 414 |

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.

**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 fT3. Based on data obtained by WEL-LISA, 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

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| **Sex, age** | **Units pmol/l** | |
| Lower limit | Upper limit |
| Healthy donors | 2.5 | 8.5 |

**PERFORMANCE CHARACTERISTICS**

Analytical specificity / Cross reactivity

|  |  |
| --- | --- |
| **Analyte** | **Cross-reactivity, % wt/wt** |
| L-T3 | 100 |
| DT3 | 100 |
| L-Thyroxin | 0.01 |
| D-Thyroxin | 0.01 |

**ANALYTICAL SENSITIVITY**

Sensitivity of the assay was assessed as being 0,5 pmol/l.

**LINEARITY**

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

**RECOVERY**

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

**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

PIN/FT3/18/00



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