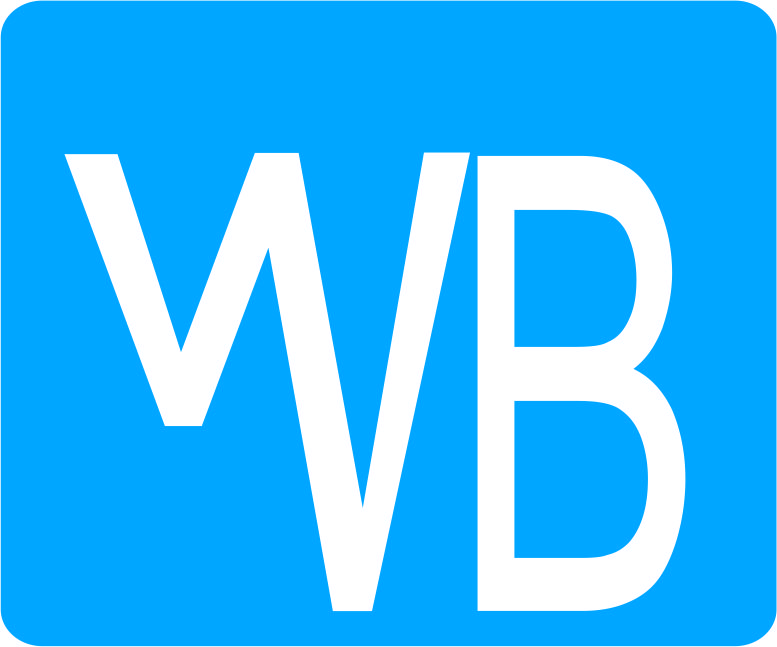
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**WELDON BIOTECH**

***Inspiration for Life Science***

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| **Wel-*Lisa* Triiodothyronine (T3) ELISA** |

**Cat.No.TT3-101WB (96 Tests)**

**INTENDED USE**

The Wel-Lisa T3, A solid phase enzyme immunoassay for the quantitative determination of triiodothyronine in blood serum or plasma. This kit is designed for measurement of triiodothyronine in blood serum or plasma. For possibility of use with others sample types, Please refer to Application notes (on request).The kit contains reagents sufficient for 96 determinations and allows analyzing 42 unknown samples in duplicates.

**SUMMARY AND EXPLANATION**

Thyroid hormones thyroxin (T4) and 3,5,3’-triiodothyronine (T3) exert regulatory influences on growth,differenciation,cellular metabolism and development of skeletal and organ system. 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 thyroidgland. 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 must 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 concentration of T3 are found in initial stage of hyperthyroidism , acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates. Decreased concentration of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis , after intake of androgens, dexamethasone, salycilates .

**PRINCIPLE OF THE TEST**

The Wel-Lisa T3 test is based on competition enzyme immunoassay principle. Tested specimen is placed into the micro wells coated by specific rabbit polyclonal to T3 antibodies simultaneously with conjugated T3 peroxidase. T3 from the specimen compets with the conjugated T3 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 450nm. 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.

1. INFECTION HAZARD: There is no available test method that can absolutely assure that Hepatitis B and C virus,HIV-1/2 , or other infection agents or not present in the reagents of this kit.All human products,including patient sample ,should be considerd potentially infection,handling and disposal should be in accordance with the produced defined by an appropriate national biohazard safety guidelines for regulations.
2. Avoid contact with stop solution containing 5.0% H2So4 . It may cause skin irritation and burns.
3. Wear disposal latex gloves when handling specimens and reagents. Microbial
4. contamination of reagents may give false results.
5. Do not use the kit beyond the expiration date.
6. All indicated volume have to be performed according to the protocol . Optimal test result are only obtained when using calibrated pipetts and microplate readers.
7. Do not smoke, eat, or drink or apply cosmetic in the areas in where specimens or kit reagents are handled.
8. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations .
9. Do not mix reagents from different lots.
10. Replace caps or reagents immediately. Do not swap caps.
11. Do not pipette regents by mouth.
12. Specimens must not contain any AZIDE compounds-they inhibit activity of peroxidase.
13. Material safety data sheet for this product is available upon request directly from WELDON BIOTECH PVT. LTD.
14. The Material safety data sheet fit the requirements of EU Guideline 91/155 EC.

**KIT COMPONENTS**

**Contents of the kit**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.N.** | **Symbol** | **Description** | | **Qty** | **Unit** | **Colour code** | **Stability of opened/**  **Diluted components** |
| 01 | SORB MTP | T3 EIA strips, 8х12 wells | Polystyrene microwells coated with rabbit polyclonal to T3 | 1 | No. | -------- | Until exp. date |
| 02 | CAL 1-5 | Calibrator set,  0.8 ml each. The set contains 5 calibrators: 0; 0.75;1;5;7.5;15 nmol/l | Human triiodothyronine diluted in tris buffered preservative – 0.01% Bronidox L, 0.01% 2-Methyl -4-isothiazolin-3-one-hydrochloride ;also contains blue dye | 5 | No. | blue  (C1 -colourless) | Until exp. date |
| 03 | CONTROL | Control serum (0.8ml) | Dilution of preselected human serum, with high content of triiodothyronine with preservative – 0.01% Bronidox L, 0.01% 2-Methyl -4-isothiazolin-3-one-hydrochloride, colourless | 1 | No. | colourles | Until exp. date |
| 04 | CONJ 2X | Conjugate concentrate,  7 ml | Aqueous solution of T3 coupled with horse radish peroxidase diluted on phos-phate buffered saline with casein from bovine milk and detergent(Tween-20),contains 0.1% phenol as preservative and blue dye | 1 | No. | Blue | Concentrate - until exp. date Diluted – 1 day at 2-8 ̊C |
| 05 | DIL CONJ | Conjugate dilution buffer, 7 ml | Aqueous Tris-buffered BSA solution, preservative -0.01% Bronidox L, 0.01% 2-Methyl -4-isothiazolin-3-one-hydrochloride;contains blue dye | 1 | No. | blue | Until exp. date |
| 06 | SUBS TMB | Substrate solution, 14ml | ready –to-use single-component tetramethylbenzidine (TMB) solution. | 1 | No. | Colourless | Until exp. date |
| 07 | BUF WASH 26X | Washing solution  Concentrate 26X , 22ml | Aqueous solution of sodium chloride and detergent(Tween20),contains proclin300 as a preservative | 1 | No. | colourless | Concentrate - until exp. date Diluted washing solution – 45 day s at 2-8 ̊C or 15 days at RT |
| 08 | STOP | Stop solution , 14 ml | 5.0℅ vol/vol solution of sulphuric acid | 1 | No. | colourless | Until exp. date |
| 09 | Noo3 | Plate sealing tape | ------------------ | 2 | No. | ---------- | N/A |
| 10 | K211I | Instruction T3 EIA | ------------- | 1 | No. | ------------ | N/A |
| 11 | K211Q | QC data sheet T3 EIA | ------------ | 1 | No. | ------ | N/A |

**EQUIPMENT AND MATERIAL REQUIRED**

**BUT NOT PROVIDED:**

1. Distilled or deionized water
2. Automatic or semiautomatic multichannel micropipettes,100-250 µl,is useful but not essential;
3. Calibrated micropipettes with variable volume,range volume 25-250 µl;

4. Dry thermostat for 37±2ºC;

5. Calibrate microplate photometer with 450nm wavelength and OD measuring range 0-3.0 .

**STORAGE AND STABILITY:**

1. Store the kit at +2 to + 8° C upon receipt until the expiration date.
2. After opening the pouch keep unused 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 micro strips) 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.
* Prepare washing solution from the concentrate BUFWASH26X by 26 dilutions in distilled water.
* Conjugate concentration to be dilute Conjugate Dilution in 1:1 ratio.

(Example: fore 16 wells Conjugate concentration 850μl + Conjugate Dilution 850μl, mix slightly Excess)

**PROCEDURAL NOTE:**

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

**ASSAY FLOW CHART**

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

**ALTERNATIVE UNITS**

1 nmol/l = 0.65 ng/ml

**ASSAY PROCEDURE**

1. Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators CAL 1–5 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS**.**
2. Prepare working conjugate solution by dilution of conjugate concentrate 2 fold by conjugate dilution buffer.

**ATTENTION :** working conjugate solution is unstable and should not be stored ! Prepare the volume required for actual assay run.

1. Pipet 25 µl of calibrators CAL 1–5, control samples CONTROL and unknown samples into the wells.
2. Dispense 100 µl of working conjugate solution into the wells. Cover the wells by plate adhesive tape (included into the kit).
3. Incubate 60 minutes at 37 °C.
4. Prepare washing solution by 26X dilution of washing solution concentrate (BUF WASH 26X) by distilled water. Wash the strips 5 times.
5. Dispense 100 µl of SUBS TMB into the wells.
6. Incubate 10-20 minutes at +18…+25 °C.
7. Dispense 100 µl of STOP into the wells.
8. Measure OD (optical density) at 450 nm.
9. Set photo meter blank on air.
10. 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. More over 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.

**CALCULATION OF RESULTS:**

Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.Plot a calibration curve on graph paper: OD versus triiodothyronine concentration. Determine thecorresponding concentration of 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.Below is presented a typical example of a standard curve with the Weldon. Not for calculations!

|  |  |  |
| --- | --- | --- |
| **Calibrators** | **Value** | **Absorbance Units**  **(450 nm)x1000** |
| CAL 1 | 0 nmol/l | 2552 |
| CAL 2 | 0.75 nmol/l | 2003 |
| CAL 3 | 1.5 nmol/l | 1614 |
| CAL 4 | 7.5 nmol/l | 714 |
| CAL 5 | 15 nmol/l | 398 |

**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 T3. Based on data obtained by Weldon, the following normal range is recommended (see below).

**NOTE:** The patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high tittered 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 nmol/l** | | | | **Units alternative ng/ml** | | |
| **Lower Limit** | | **Upper Limit** | | **Lower Limit** | | **Upper Limit** |
| **Healthy donors** | **1.2** | | **3.2** | | **0.8** | | **2.1** | |

**PERFORMANCE CHARACTERISTICS**

Analytical specificity/Cross reactivity.

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

**ANALYTICAL SENSITIVITY:**

Sensitivity of the assay was assessed as being 0.2 nmol/l.

**LINEARITY:**

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

**RECOVERY:**

Recovery was estimated by assaying 5 mixed samples with known 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,c h 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

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