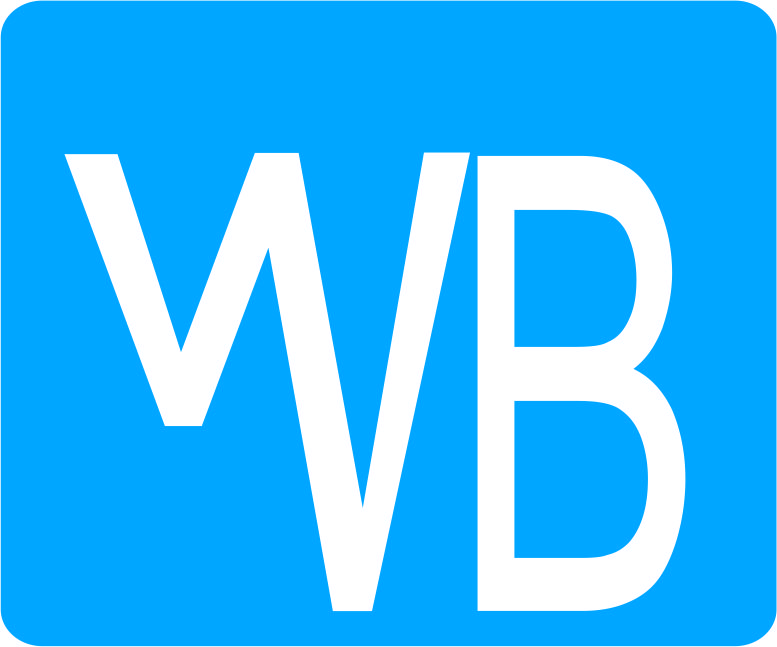
**WELDON BIOTECH**

***Inspiration for Life Sciences***

|  |
| --- |
| **Wel-*Lisa* Thyroxin Hormone (TT4) ELISA** |

**Cat.No.TT4-102WB (96 Tests)**

**INTENDED USE**

A solid-phase enzyme immunoassay for the quantitative determination of thyroxin in human serum or plasma. This kit is designed for measurement of thyroxinin 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 42 unknown samples in duplicates.

**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 T4 is generally accepted as an index of thyroid function which provide enough information to differentiate between hyper-, hypo- and euthyroidism.

Elevation of total T4 is found in hyperthyroidism, in patients with tumours of pituitary gland, in subjects with elevated TBG level (pregnancy, acute or chronic active hepatitis, estrogen-secreting tumours or estrogen intake, hereditary elevation of TBG), in patients taking oral contraceptives, heroin, methadone, thyroid preparations, TSH, thyroliberin.

Low total T4 is found in hypothyroidism, in patients with panhypopituitarism, in subjects with low TBG level (acromegaly, nephritic syndrome, hypoproteinemia, chronic liver diseases, androgen-secreting tumours, hereditary reduction), in patients taking aminosalicylic and acetylsalicylic acids, cholestyramine, reserpine, potassium iodide, triiodothyronine.

**PRINCIPLE OF THE TEST**

This test is based on competition enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to T4-antibodies simultaneously with conjugated T4-peroxidase. T4 from the specimen competes with the conjugated T4 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 ofreagents 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. Material Safety Data Sheet for this product is available upon request directly from *WEL-LISA* Co., Ltd.
15. The Material Safety Data Sheet fit the requirements of EU Guideline 91/155 EC.

**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:**

1. Store the kit at +2 to + 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until the expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

**KIT COMPONENTS**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No.** | ***Symbol*** | ***Description*** | | ***Qty*** | ***Units*** | ***Colour code*** | ***Stability of opened/diluted components*** |
| 1 | SORB MTP | T4 EIA strips, 8х12 wells | polystyrene microwells coated with murine monoclonal to T4 | 1 | No. | -- | until exp.date |
| 2 | CAL 1 - 5 | Calibrator set, 0.8 ml each. The set contains 5 calibrators: 0; 32; 64; 160; 320 nmol/l | human thyroxin diluted in a preselected human serum preservative - 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains red dye | 5 | No. | red (C1 - colourless) | 2 months |
| 3 | CONTROL | Control serum(0.8 ml) | dilution of preselected human serum, with high content of thyroxin 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 T4 coupled with horseradish peroxidase diluted on phosphate buffered solution preservative - 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride and red dye | 1 | No. | red | until exp.date |
| 5 | SUBS TMB | Substrate solution,14 ml | ready-to-use single-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 | K212I | Instruction T4 EIA | - | 1 | No. | - | N/A |
| 10 | K212Q | QC data sheet T4 EIA | - | 1 | No. | - | N/A |

**SPECIMEN COLLECTION AND STORAGE:**

This kit is intended for use with serum or plasma (ACD-orheparinized).Grosslyhemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored for up to 48 hours at +2…+8°Cbeforetesting.

For a longer storage, the specimens should be frozen at-20°C or lower. Repeated freezing/thawing should be avoided.

**TEST PROCEDURE**

**REAGENTS 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 overtaxing 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.

**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

**ASSAY PROCEDURE**

1. Put the desired number of microstrips into the frame; allocate 14 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. Pipet 25 µl of calibrators CAL 1–5, control samples CONTROL and unknown samples into the wells.
3. Dispense 100 µl of CONJ HRP into the wells.
4. Cover the wells by plate adhesive tape (included into the kit).
5. Incubate 60 minutes at 37 °C.
6. Prepare washing solution by 26X dilution of washing solution concentrate (BUF WASH 26X) by distilled water. Wash the strips 5 times.
7. Dispense 100 µl of SUBS TMB into the wells.
8. Incubate 10-20 minutes at +18…+25 °C.
9. Dispense 100 µl of STOP into the wells.
10. Measure OD (optical density) at 450 nm.
11. Set photo meter blank on air.
12. Apply point-by-point method for data reduction.

**HANDLING NOTES:**

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

**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 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 total Thyroxin concentration. Determine thecorresponding concentration of total Thyroxin 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.Below is presented a typical example of a standard curve with the Wel-Lisa. Not for calculations

|  |  |  |
| --- | --- | --- |
| **Calibrators** | **Value** | **Absorbance at**  **(450 nm) x1000** |
| CAL 1 | 0 nmol/l | 3020 |
| CAL 2 | 32 nmol/l | 2196 |
| CAL 3 | 64 nmol/l | 1060 |
| CAL 4 | 160 nmol/l | 435 |
| CAL 5 | 320 nmol/l | 205 |

**EXPECTED VALUES**

Therapeutically 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 Weldon the following normal range is recommended (see below).

**NOTE:**

The patients that have received murine monoclonal antibodies for radio imaging or immuno therapy 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** | | | **Units alternative** | |
| **nmol/l** | | | **μg/dl** | |
| **Lower Limit** | | **Upper Limit** | **Lower Limit** | **Upper Limit** |
| **Healthy Donors** | 60 | | 160 | 4.7 | 12.4 |
| **Males** | | | | | |
| **≥ 61Years** | 60 | | 129 | 4.7 | 10.0 |
| **Females** | | | | | |
| **≥ 61Years** | | 70 | 135 | 5.4 | 10.5 |
| **Children** | | | | | |
| **1.5 Years** | | 90 | 190 | 7.0 | 14.7 |
| **6-10 Years** | | 83 | 170 | 6.4 | 13.2 |
| **>10 Years** | | 60 | 160 | 4.7 | 12.4 |

**PERFORMANCE CHARACTERISTICS**

Analytical specificity/Cross reactivity.

|  |  |
| --- | --- |
| **Analyte** | **Cross-reactivity, %wt/wt** |
| L-Thyroxin | 100 |
| D-Thyroxin | 30 |
| T3 | 0.5 |

**ANALYTICAL SENSITIVITY:**

Sensitivity of the assay was as secedes being 3 nmol/l

**LINEARITY:**

Linearity was checked by assaying dilution series of 5 samples with different Thyroxin concentrations. Linearity percentages obtained ranged within 90 to110%.

**RECOVERY:**

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

**LITERATURE:**

1. Helfand M et al. Screening for thyroid disease. Ann Intern Med 1990; 112:840.

2. Chopra, I.J. et al. A Radioimmunoassay of Thyroxine. J. Clinical EndocrinoL 1971; 33:865.

3. Young, D.S. et al. Effects of Drugs on Clinical Laboratory Tests. Clinical Chemistry 1975; 21: 3660.

4. Sterling, L. Diagnosis and Treatment of Thyroid Disease, Cleveland , CRC Press, P. 19 51 (1975).

5. Surks M.I. et al. American Thyroid Association guidelines for use of laboratory tests in thyroid disorders. JAMA 1990; 263:1529

PIN/TT4/18/00



**Weldon Biotech (India) Private Limited**

Plot No.: 31, Sector- 2, I.I.E., SIDCUL, Haridwar, Uttarakhand. Pin- 249403,

Tel.: 01334-239441-42

Ph. No. 08006553470

Email: [info@weldonbiotech.com mfg@weldonbiotech.com](mailto:info@weldonbiotech.com%20%20mfg@weldonbiotech.com),

Website: www.weldo