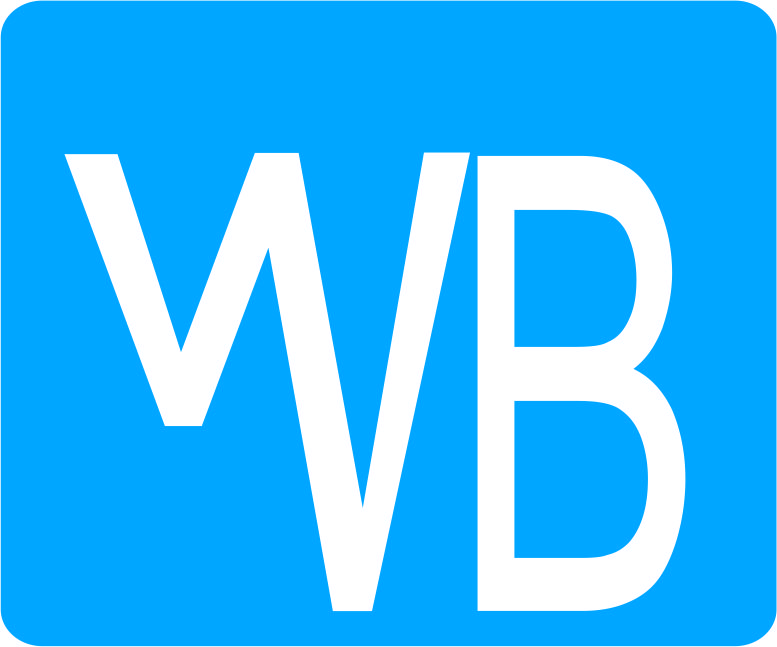
**WELDON BIOTECH**

***Inspiration for Life Sciences***

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| **Wel-*Lisa* Thyroid Stimulating Hormone (TSH) ELISA** |

**Cat.No.TSH-105WB (96 Tests)**

**INTENDED USE**

The Wel-Lisa TSH, A solid phase enzyme immunoassay for the quantitative determination of TSH in blood serum or plasma. This kit is designed for measurement of TSH 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 41unknown samples in duplicates.

**SUMMARY AND EXPLANATION**

Thyroid stimulating hormone (TSH) is a glycoprotein with molecular weightca.30 KD which is secreted by hypophysis. A molecule of TSH consists of two none covalently bound sub units: α-and β-HCG. β-subunit determines biological activity and immunological specificity of TSH.

TSH stimulates thyroid gland to secrete thyroid hormones. TSH secretion in hypophysis is controlled by a negative feedback regulation by thyroid hormones.TSH secretion is subject to circadian rhythms with highest levels seen early in the morning (6a.m.).Changes of TSH blood level during a day are not significant; never the less, if the results do not correspond with clinical status and other laboratory data, it is recommended to take and test another blood sample. Determination of TSH level in serum is recommended in the following states and conditions:

1. Diagnostics of dysfunction of the thyroid gland;
2. Hypothyroidism (TSH level is increased. The diagnosis is confirmed by low concentrations of total and freeT4 andT3.In mild subclinical forms whenT4 and T3 levels are within normal ranges, determination of TSH concentration is critical);
3. Hyperthyroidism(synthesis and secretion of TSH are inhibited);monitoring of replacement therapy;
4. Screening for inherited hypothyroidism (on day 5 after birth TSH level in blood is determined).TSH level is elevated just after birth but it comes within the normal range in several days (both in boys and in girls). Serum TSH level is elevated during pregnancy, after physical stress, in individuals with lowered blood pressure and lowered temperature. Secretion of TSH is inhibited by Cortical and Growth hormone .Low TSH levels are often seen in elderly people, in patients with chronicrenalin sufficiency, liver cirrhosis, in retardation of sexual development, in secondary amenorrhea, Cushing syndrome, acromegaly.

In a present test system-chain specific monoclonal antibodyXTB78 is used as capture reagent; enzyme-labeled (Fab2)-fragment of another β-chain specific monoclonal antibodyXTB11 is used as tracer. This combination enables to minimize both cross-reactive reactions with other pituitary hormones and false positivity caused by anti-species antibodies.

**PRINCIPLE OF THE TEST**

The Wel-Lisa TSH test is based on sandwich enzyme immunoassay principle. Tested specimen is placed in to the micro wells coated by specific murine monoclonal to β chain human TSH-antibodies. Antigen from the specimen is captured by the antibodies coated microwell surface. Second antibodies murine monocnoclonal to (Fab2) fragment of β chain humanTSH,labeled with peroxidase enzyme,are addeintthmicrowells. After washing procedure, the remaining

enzymatic activity bound to the microwell surface isdetected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly 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. Potential biohazardous materials: The standards set contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. It is recommended that standards, control and serum samples be run in duplicate.
5. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.
6. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.

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| **S.N.** | **Symbol** | **Description** | | **Qty** | **Unit** | **Colour code** | **Stability of opened/**  **Diluted components** |
| 01 | SORB MTP | TSH EIA strips, 8х12wells | Polystyrene microwells coated with murine monoclonal to β chain human TSH | 1 | No. |  | Until exp. date |
| 02 | CAL 1-6 | Calibrator set,  0.8ml each, zero calibrator C1–2 ml The set contains 6 calibrators:0;0.2;1;5;10;20mIU/l | Human TSH diluted in phosphate buffered of preselected horses serum, casein solution,preservative–0.1%phenol;also contains brightred dye | 6 | No. | Red  (C1 colourless) | 2 months |
| 3 | CONTROL | Control serum(0.8ml) | Dilution of preselected human serum, with high content of TSH with casein solution;preservative–0.1% phenol, colourless | 1 | No. | colourles | 2 months |
| 4 | CONJ HRP | Conjugate,14ml | Aqueous solution of murinemonocnoclonal to (Fab2)-fragment of βchain humanTSH coupled with horse radish peroxidase diluted on phos-phatebuffe red solution with casein from bovine milk and detergent(Tween- | 1 | No. | Blue | Until exp. date |
| 5 | SUBS  TMB | Substrate solution, 14ml | Ready –to-usesingle-componentteramethylbenzidine (TMB) solution. | 1 | No | colourless | Untill exp. date |
| 6 | BUF  WASH  26X | Washing solution  Concentrate26X,22ml | Aques solution of sodium chloride and detergent(Tween20),contain proclean300 as a preservative | 1 | No | Colourless | Concentrate until exp. date Dioluted waqshing solution-45day sat 2-8 ̊ cor15 days satRT |
| 7 | STOP | Stop solution, 14ml | 5.0℅ vol/vol solution of sulphuric acid | 1 | No | colourless | Until exp. date |
| 8 | N003 | Platesealing tape |  | 2 | No | colourless | Until exp. date |
| 9 | K201ICEIR | Instruction TSHEIA, English |  | 1 | No |  | N/A |
| 10 | K201Q | QC data sheet TSH 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:**

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.

**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 1–6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS**.**
2. If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using (zero calibrator). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
3. Dispense 100 µl of CONJ HRP into the wells.
4. Pipet 50 µl of calibrators CAL 1–6, control samples CONTROL and unknown samples into the wells. 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.

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| **Calibrators** | **Value** | **Absorbance at**  **(450 nm)** |
| CAL 1 | 0 mIU/L | 0.07 |
| CAL 2 | 0.2 mIU/L | 0.12 |
| CAL 3 | 1 mIU/L | 0.23 |
| CAL 4 | 5 mIU/L | 0.74 |
| CAL 5 | 10 mIU/L | 1.34 |
| CAL 6 | 20 mIU/L | 2.18 |

1. Incubate 10-20 minutes at +18…+25 °C.
2. Dispense 100 µl of STOP into the wells.
3. Measure OD (optical density) at 450 nm.
4. Set photo meter blank on first calibrator.
5. 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 TSH concentration. Determine thecorresponding concentration of total TSH 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 ton on-linear shape of curve.Below is presented a typical example of a standard curve with the Weldon. Not for calculations.

**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 TSH. 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.

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| **Sex age** | **Units mIU/l** | |
| **Lower Limits** | **Upper Limits** |
| **Healthy donors** | **0.3** | **4.0** |

**PERFORMANCE CHARACTERISTICS**

Analytical specificity/Cross reactivity.

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| **Analyte** | **Cross-reactivity, %wt/wt** |
| HCG | <0.1 |
| LH | <0.1 |
| FSH | <0.1 |

**ANALYTICAL SENSITIVITY:**

Sensitivity of the assay was as secedes being 0.04 mIU/l.

**LINEARITY:**

Linearity was checked by assaying dilution series of 5samples with different TSH 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%.

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PIN/TSH/18/00

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