

Read entire protocol before use.

## TESTO-Elisa

### I. INTENDED USE

Enzyme Immunoassay for the *in vitro* quantitative measurement of human Testosterone (TESTO) in serum.

### II. GENERAL INFORMATION

- A. Name: Bio-Line **Testosterone-Elisa** Kit
- B. Catalogue number: **BL-12-E**: 96 tests
- C. Manufactured by: Bio-Line S.A.  
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
- D. For technical assistance or ordering information contact:  
Tel: +32-2-736.62.18. Fax: +32-2-742.13.15.

### III. CLINICAL BACKGROUND

#### A. Biological activity

Testosterone is a C-19 steroid hormone (molecular weight: 288 Da) which is produced from androstenedione in the testes, adrenals and ovaries. Testosterone is a precursor along with androstenedione of the estrogen steroids.

#### B. Clinical applications

- **Clinical significance of testosterone level** : Source of testosterone :
  - Women : Ovary, adrenal cortex, peripheral tissues (by conversion of 50-60% other steroids).
  - Men : Testes > 90%, adrenal cortex, peripheral tissues.
- **Clinical diseases with high level of testosterone** :
  - Women : Hirsutism and virilization, polycystic ovary syndrome, congenital adrenal hyperplasia (with 17OH-PROG), tumors of adrenal and ovarian origin, breast cancer.
  - Men : Disease of the hypothalamic pituitary unit, some malignant testicular tumors, congenital adrenal hyperplasia, prostate cancer.
- **Clinical diseases with low level of testosterone** :  
Primary or secondary hypogonadism, Klinefelter's syndrome, other chromosomal alteration, hypopituitarism, enzymatic defects, orchidectomy and cryptorchidism, testicular feminization, hepatic cirrhosis, some autoimmune diseases for example : Sjögren's syndrome, systemic lupus.
- **Other domains for measurement of testosterone level** :
  - *In vitro* fertilization : the women with high response to gonadotropin have a significant increase in testosterone.
  - Parameter of the prepuberty and puberty.
  - Determination of foetal sex in amniotic fluid.
  - Free testosterone is significantly raised in both male and female acne sufferers.
  - Follow up of cancer and in pathological situations; low testosterone syndrome.

#### IV. PRINCIPLES OF THE METHOD


The Bio-Line TESTO-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on a microtiterplate. A fixed amount of testosterone labelled with horseradish peroxidase (HRP), compete with unlabelled testosterone present in the calibrators, controls and samples for a limited number of binding sites on a specific antibody.

After 2 hours incubation at room temperature, the microtiterplate is washed to stop the competition reaction.

The revelation solution (TMB – H<sub>2</sub>O<sub>2</sub>) is added and incubated for 30 min. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is inversely proportional to the TESTO concentration.

A calibration curve is plotted and TESTO concentration in samples is determined by interpolation from the calibration curve.

#### V. REAGENTS PROVIDED

Reagents	96 tests Kit	Color Code	Reconstitution			
 Microtiterplate with 96 anti TESTO coated wells	96 wells	blue	Ready for use			
<table border="1" data-bbox="65 741 292 792"> <tr> <td>Ag</td> <td>HRP</td> <td>CONC</td> </tr> </table> Conjugate: HRP labelled TESTO (HPLC grade) in TRIS-maleate buffer with bovine gelatin and thymol	Ag	HRP	CONC	1 vial 1 ml	red	Dilute 0.2 ml in 1 vial of conjugate buffer
Ag	HRP	CONC				
<table border="1" data-bbox="65 898 236 927"> <tr> <td>CONJ</td> <td>BUF</td> </tr> </table> Conjugate buffer: Phosphate buffer with bovine gelatin and thymol	CONJ	BUF	3 vials 21 ml	red	Ready for use	
CONJ	BUF					
<table border="1" data-bbox="97 1010 225 1039"> <tr> <td>CAL</td> <td>0</td> </tr> </table> Zero calibrator in human serum and thymol	CAL	0	1 vial lyophilized	yellow	Add 1.0 ml distilled water	
CAL	0					
<table border="1" data-bbox="97 1122 225 1151"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrator N = 1 to 5 (see exact values on vial labels) in human serum and thymol	CAL	N	5 vials lyophilized	yellow	Add 0.5 ml distilled water	
CAL	N					
<table border="1" data-bbox="65 1279 256 1308"> <tr> <td>WASH</td> <td>SOLN</td> <td>CONC</td> </tr> </table> Wash Solution (Tris-HCl)	WASH	SOLN	CONC	1 vial 10 ml	brown	Dilute 200 x with distilled water (use a magnetic stirrer).
WASH	SOLN	CONC				
<table border="1" data-bbox="65 1368 240 1397"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls - N = 1 or 2 in human serum with thymol	CONTROL	N	2 vials lyophilized	silver	Add 0.5 ml distilled water	
CONTROL	N					
<table border="1" data-bbox="65 1480 256 1509"> <tr> <td>CHROM</td> <td>TMB</td> <td>CONC</td> </tr> </table> Chromogen TMB (Tetramethylbenzidine) in Dimethylformamide	CHROM	TMB	CONC	1 vial 1 ml	green	Dilute 0,1 ml into 1 vial of substrate buffer
CHROM	TMB	CONC				
<table border="1" data-bbox="65 1615 236 1644"> <tr> <td>SUB</td> <td>BUF</td> </tr> </table> Substrate buffer: H <sub>2</sub> O <sub>2</sub> in acetate / citrate buffer	SUB	BUF	3 vials 21 ml	white	Ready for use	
SUB	BUF					
<table border="1" data-bbox="65 1727 256 1756"> <tr> <td>STOP</td> <td>SOLN</td> </tr> </table> Stop solution: H <sub>2</sub> SO <sub>4</sub> 1.8N	STOP	SOLN	1 vial 6 ml	black	Ready for use	
STOP	SOLN					

**Note:** 1. Use the zero calibrator for sample dilutions.

#### VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

- High quality distilled water
- Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 500 µl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
- Washer for microtiterplates
- Microtiterplate reader capable of reading at 450 nm and 650 nm (or 630 nm)

#### VII. REAGENT PREPARATION

- Calibrators** : Reconstitute the zero calibrator with 1.0 ml distilled water and the other calibrators with 0.5 ml distilled water.
- Controls** : Reconstitute the controls with 0.5 ml distilled water.
- Working TESTO-HRP conjugate** : dilute 0.2 ml of the concentrated TESTO-HRP conjugate into one of the vials of conjugate buffer. Extemporaneous preparation is recommended.
- Working Wash solution** : Prepare an adequate volume of Working Wash solution by adding 199 volumes of distilled water to 1 volume of Wash Solution (200x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.
- Revelation Solution**: pipette 0.1 ml of the chromogen TMB into one of the vials of substrate buffer (H<sub>2</sub>O<sub>2</sub> in acetate/citrate buffer). Extemporaneous preparation is recommended.

#### VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators and controls are stable for 1 week at 2 to 8°C. For longer storage periods, aliquots should be made and kept at -20°C. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- The Working TESTO-HRP conjugate is stable for 4 hours at room temperature or for 24 hours at 2-8°C, avoid direct sunlight.
- The freshly prepared revelation solution is stable, before use, for maximum 15 minutes at room temperature and must be discarded afterwards.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

#### IX. SPECIMEN COLLECTION AND PREPARATION

- Serum must be kept at 2 - 8°C.
- If the test is not run within 24 hours, storage in aliquots at -20°C is recommended. Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- Do not use haemolysed samples.

#### X. PROCEDURE

- Handling notes**  
Do not use the kit or components beyond expiry date.  
Do not mix materials from different kit lots.  
Bring all the reagents to room temperature prior to use.  
Thoroughly mix all reagents and samples by gentle agitation or swirling.  
Perform calibrators, controls and samples in duplicate. Vertical alignment is recommended.  
Use a clean plastic container to prepare the Wash Solution.  
In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample.  
For the dispensing of the Revelation Solution and the Stop Solution avoid pipettes with metal parts.

High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in section XIII paragraph E (Time delay).

Prepare a calibration curve for each run, do not use data from previous runs.

The chromogenic solution should be colourless. If a dark blue colour develops within a few minutes after preparation, this indicates that the preparation is unusable and must be discarded.

Dispense the Revelation Solution within 15 minutes following the washing of the microtiterplate.

During incubation with Revelation Solution, avoid direct sunlight on the microtiterplate.

#### B. Procedure

- Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8 °C.
- Secure the strips into the holding frame.
- Pipette 50 µl of each Calibrator, Control and Sample into the appropriate wells.
- Pipette 200 µl of TESTO-HRP conjugate into all the wells.
- Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
- Aspirate the liquid from each well.
- Wash the plate 3 times by:
  - Dispensing 0.4 ml of Wash Solution into each well
  - Aspirating the content of each well
- Pipette 200 µl of the freshly prepared revelation solution into each well within 15 minutes following the washing step.
- Incubate the microtiterplate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
- Pipette 50 µl of Stop Solution into each well.
- Read the absorbencies at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section XI.

#### XI. CALCULATION OF RESULTS

- Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
- Calculate the mean of duplicate determinations.
- Calculate for each calibrator, control and sample:

$$B/B_0(\%) = \frac{OD(\text{Calibrator, Control or Sample})}{OD(\text{Zero Calibrator})} \times 100$$

- Using either linear-linear or semi-logarithmic graph paper, plot the (B/B<sub>0</sub>(%)) values for each calibrator point as a function of the TESTO concentration of each calibrator point. Reject obvious outliers.
- Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
- By interpolation of the sample (B/B<sub>0</sub>(%)) values, determine the TESTO concentrations of the samples from the calibration curve.

#### XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

TESTO-ELISA		OD units	B/B <sub>0</sub> (%) values
Calibrator	0 ng/ml	1.805	100
	0.12 ng/ml	1.599	88.6
	0.40 ng/ml	1.309	72.5
	1.40 ng/ml	0.948	52.5
	4.7 ng/ml	0.587	32.5
	13.0 ng/ml	0.359	19.9

#### XIII. PERFORMANCE AND LIMITATIONS

##### A. Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average OD at zero binding, was 0.05 ± 0.02 ng/ml.

##### B. Specificity

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
DiHydroTestosterone	0.61
Androstenedione	0.76
17-β-Estradiol	0.023
17-OH-Progesterone	0.018
Progesterone	0.035
DHEA	0.003
DHEA-sulphate	0.001
Cortisol	0.002
Danazol	0.003
Ethinylestradiol	<0.001
Ethisterone	0.0018

##### C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	N	<X> ± SD (ng/ml)	CV (%)	Serum	N	<X> ± SD (ng/ml)	CV (%)
A	20	0.4 ± 0.03	8.5	D	10	0.83 ± 0.06	7.3
B	20	2.45 ± 0.17	6.8	E	10	5.4 ± 0.37	6.9
C	20	4.7 ± 0.34	6.2	F	10	8.4 ± 0.53	6.4

SD : Standard Deviation; CV: Coefficient of variation

##### D. Accuracy

###### RECOVERY TEST

Sample	Added TESTO (ng/ml)	Recovered TESTO (ng/ml)	Recovery (%)
Serum	5.5	5.7	97
	4.1	3.9	95
	2.5	2.8	112
	1.6	1.8	112
plasma	5.5	5.7	104
	4.4	4.4	100
	2.7	2.8	104
	1.8	1.8	102

###### DILUTION TEST

Sample	Dilution	Theoretical Concent. (ng/ml)	Measured Concent. (ng/ml)
Serum 1	1/1	-	4.65
	1/2	2.33	2.62
	1/4	1.16	1.24
	1/8	0.58	0.63
	1/16	0.29	0.33

Sample was diluted with zero calibrator.

##### Conversion factor :

From ng/ml to nmol/L : x 3.47

From nmol/L to ng/ml : x 0.288

To the best of our knowledge, no international reference material exists for this parameter.

The concentrations of the calibrators are determined with the ID-GC/MS reference method.

##### F. Correlation with reference method

Serum samples from 21 women and 17 men, ranging from 0.15 to 8.9 ng/ml, were tested with the TESTO-ELISA and a titrated

reference method. The linear regression shows a good correlation between these two methods:

$$\text{TESTO-ELISA} = 1.03 (\text{H}^3 \text{ reference}) + 0.19 \quad r=0.96 \quad n=38$$

#### XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

#### XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Sample	n	Mean (ng/ml)	Range (ng/ml)
Females	50	0.46	0.1 – 1.1
Males	50	6.6	2.6 – 13.5

#### XVI. PRECAUTIONS AND WARNINGS

##### Safety

For *in vitro* diagnostic use only.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with all reagents, Stop Solution contains H<sub>2</sub>SO<sub>4</sub>, the chromogen contains TMB in Dimethylformamide, Substrate buffer contains H<sub>2</sub>O<sub>2</sub>. In case of contact, wash thoroughly with water.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

#### XVII. BIBLIOGRAPHY

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#### XVIII. SUMMARY OF THE PROTOCOL

	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)
Calibrators (0-5) Controls, Samples TESTO-HRP conjugate	50 - 200	- 50 200
Incubate for 2 hours at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash Solution and aspirate.		
Revelation Solution	200	200
Incubate for 30 min at room temperature with continuous shaking at 700 rpm.		
Stop Solution	50	50
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm)		

Bio-Line Catalogue Nr : BL-12-E	Version : 050215-BL	Revision nr : 2
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