Life Technologies™
Your Molecular & Cell Technology Partner

Canine Testosterone Kit ELISA Kit

to determine Canine Testosterone in Serum, Blood Plasma, Saliva, Urine,

And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTaC4500EA

Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Toll Free: 1800-120-2434
Mobile: +91-98105-21400 | Fax: +91-11-42208444
customerservice@lifetechindia.com

INTENDED USE

This kit is used to assay Canine Testosterone in the sample of serum, blood plasma, saliva, urine, and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.01

PRINCIPLE

This ELISA Kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate microtiter plate wells with an antibody specific for testosterone and Horseradish Peroxidase (HRP) conjugated testosterone. The competitive inhibition reaction is launched between with HRP labeled testosterone and unlabeled testosterone with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of testosterone in the sample. The color development is stopped and the intensity of the color is measured.

MATERIALS SUPPLIED IN THIS KIT

Testosterone microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with the goat-anti-rabbit antibody.

Testosterone standard: Testosterone in a buffered protein base with preservatives, liquid.

HRP conjugated testosterone: liquid.

Testosterone detect antibody: Antibody specific for testosterone, liquid.

HRP substrate A: Urea hydrogen peroxide solution.

HRP substrate B: TMB (Tetramethyl-benzidine) solution.

Stop solution: 2 mol/L sulfuric acid.

Wash buffer: PBS with 0.5% Tween-20, 20× liquid.

Plate covers: 2

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips.
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature.
2. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8 °C.
3. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
4. All samples and all discard generated should be disposed as per local rules.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

PRECISION

Intra-assay Precision (Precision within an assay)

Four Canine samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)<15%.

Inter-assay Precision (Precision between assays)

Three Canine samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)<15%.

Recovery

The recovery of testosterone spiked to different levels in samples throughout the range of the assay in various matrices was evaluated. The recovery ranged from 85% to 115% with an overall mean recovery of 100%.

Sensitivity

The minimum detectable dose (MDD) of testosterone is typically less than 0.1 ng/ml. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This Canine Testosterone ELISA Kit has high sensitivity and excellent specificity for detection of Testosterone. No significant cross-reactivity or interference between Testosterone and analogues was observed. This Kit has been checked for determining Testosterone concentration from varied species such as bovine, chicken, fish, goat, human, rabbit, rat, sheep and swine in addition to canine.

SAMPLE PREPARATION

1. Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).
2. After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20 °C. Avoid repeated freeze-thaw cycles.
3. Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.

4. Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.
5. Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow a similar procedure.
6. Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.
7. Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8 °C. Add few mls of PBS (pH 7.4) and then homogenize the sample thoroughly by hand or by homogenizer. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.

2. Set a Blank well without any solution.

3. Add 50 µl of Standard or Sample per well. Standard need test in duplicate.

4. Add 50 µl of HRP-conjugate to each well (not to Blank well), then 50 µl detect antibody to each well. Mix well and then incubate for 1 hour at 37 °C.

5. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (250 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 10 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

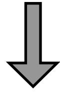
6. Add 50 µl of Substrate A and 50 µl of Substrate B to each well, mix well. Incubate for 15 minutes at 37 °C. Keeping the plate away from drafts and other temperature fluctuations
in the dark.

7. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.

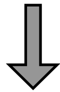
8. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

PROTOCOL SUMMARY

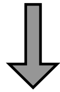
Set a Blank well without any solution. Add 50µL of standards & samples.



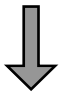
Add 50µL HRP-Conjugate and 50µL detect antibody to each well except blanks. Incubate for 60 minutes at 37°C.



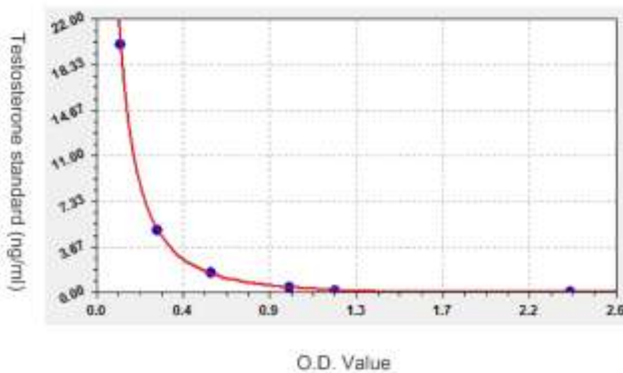
Wash the plate three times. Add Substrate Solution A and B. Incubate for 15 minutes at 37 °C for color development and add 50µL stop solution.



Read the OD value at 450 nm within 10 minutes.



Plot and Calculate.



Standard Testosterone (ng/ml)	Optical Density (450 nm)	Average
0	2.338	2.362
	2.386	
0.1	1.249	1.1965
	1.144	
0.4	1.016	0.967
	0.918	
1.6	0.598	0.582
	0.566	
5	0.322	0.3145
	0.307	
20	0.141	0.130
	0.119	

THIS TYPICAL CHART AND TABLE SHOWN HERE ARE FOR REFERENCE PURPOSES ONLY AND THE ACTUAL PERFORMANCE MAY VARY.

CALCULATION

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the concentration on the y-axis against the mean absorbance for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Testosterone concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

LINEARITY: To assess linearity of the assay, samples containing and/or spiked with high concentrations of Canine Testosterone were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

ASSAY RANGE： 0.1 ng/ml - 20 ng/ml.

PACKAGE SIZE： 96Tests.

SENSITIVITY： <0.1ng/ml.

VALIDITY & STORAGE: 1 Year (at 2-8°C) from the date of supply.