Mouse Testosterone
ELISA Kit

ZellBio GmbH (Germany)

Cat. No：ZB-10260I-M9648

Samples Type Validated
Mouse Serum/Plasma

For Research Use Only. Not for in Vitro Diagnosis

Please read this insert completely prior to using the product.

Important note:

Only the brochure inside the kit should be considered for the assay procedure, as it is the latest version of the insert.

Thank you for choosing ZellBio products. Please read the instructions carefully before use and check all the reagent compositions! If in doubt, please contact **ZellBio GmbH**.

ZellBio GmbH assay kit is used to **quantitative** assay **Mouse Testosterone** on the basis of the competition method. **ZellBio GmbH** ELISA kit takes **one-step** method with which solutions need minimum diluting, because we simplify the dilute process by our lab techniques. This kit is for research only and is not for use in diagnostic procedures.

Intended Use

ZellBio GmbH Mouse Testosterone determines Testosterone in the sample of Mouse's **Serum/Plasma.**

Principle of the Test

ZellBio GmbH Mouse Testosterone ELISA kit is based on the principle of competitive binding between Testosterone in the test specimen and Testosterone enzyme conjugate for a constant amount of anti-Testosterone polyclonal antibody. In the first step biotin

conjugated-anti-Testosterone antibody binds to the avidin coated wells during 60 min at RT. During this incubation, a fixed amount of HRP-labeled Testosterone competes with the endogenous Testosterone in the standards and sample for a fixed number of binding sites of the specific Testosterone antibody. Testosterone peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of Testosterone in the specimen increases. Unbound Testosterone peroxidase conjugate is then removed and the wells are washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance.

Materials supplied in the Test Kit

	Materials	96 Tests	48 Tests
1	ELISA Microplate	8 wells×12	8wells×6
2	Standards (0-16ng/mL)	7 Vials (0.5mL)	7 Vials (0.2mL)
3	Testosterone Biotin Conjugate	6mL	3mL
4	Testosterone Enzyme Conjugate	6mL	3mL
5	Concentrated Wash Buffer (50X)	20mL	20mL
6	Substrate A	6.5mL	3mL
7	Substrate B	6.5mL	3mL
8	Stop Solution	6mL	3mL
9	Instruction	1	1
10	Adhesive Sheet	2	2

Materials required but not supplied

1. Standard Microplate reader
2. Precision pipettes and Disposable pipette tips
3. Distilled water
4. Disposable tubes for Standards and samples dilution
5. Absorbent paper

Specimen requirements

1. Samples containing NaN_3 must **not be tested** as it inhibits the activity of Horse Radish Peroxidase (HRP).

2. After collecting the sample, extraction should be immediately carried out in accordance with related documents. **After extraction, experiment should be conducted immediately as well (during 24 hours, can be keep at 2-8℃). Keep the sample at -20℃ or deeper Freeze for long time. Avoid repeated freeze-thaw cycles.**

3. **Serum**: Allow the blood to clot for 10-15 minutes at room temperature. Centrifuge (at 2000-3000 RPM) for 10 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

4. **Blood plasma**: In accordance with the requirements of sample collection, EDTA or sodium citrate should be used as anticoagulation. Add EDTA or sodium citrate and mix them completely for minutes. Centrifuge (at 2000-3000 RPM) for approximately 10 minutes. Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.

Sample preparation

1. **ZellBio GmbH** is only responsible for the kit itself, but not for the samples consumed during the experiment. The user should calculate the possible amount of the samples needed in the whole test. Reserving sufficient samples in advance is recommended.

2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the kit is necessary.

3. Tissue or cell extraction samples prepared by chemical Lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

4. Due to the possibility of mismatching between antigen from other origins and antibodies used in our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.

5. Influenced by factors including cell viability, cell number or sampling time, molecular from cells culture supernatant may not be detected by the kit.

6. Grossly hemolyzed samples are not suitable for use in the assay.

7. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

Bring all reagents to room temperature (18-25℃) before use.

Standards, Biotin & HRP Conjugate: Ready to Use

Wash Buffer: Dilute 10 mL of Concentrated Wash Buffer into 490 mL of deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Note: Substrate Solution is easily contaminated. Please protect it from light. Please don't prepare the reagent directly in the Diluent vials provided in the kit. Contaminated water or container for reagent preparation will influence the result.

Washing method

Manually washing method: Washing by hand: Shake off the liquids in the wells of the ELISA plate; Lay several bibulous papers on the test bed and tapping hard the ELISA plate several times downward; then inject at least 300μl of diluted washing concentration for 1-2 minutes' soaking. Repeat this process as needed.

Automatic washing method: Washing by automatic plate washer: If there is an automatic plate washer, it should only be used in the test when you are quite familiar with its functions.

Important Note

1. Before opening the kit kept at the temperature of 2-8℃, it takes at least 30 minutes to increase naturally to room temperature. The just opened ELISA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results. After breaking the seal of ELISA coated-plate, some of the stripes used should be kept in hermetic bag.
2. The instruction must be strictly followed while the reading of ELISA reader must be set as the standard of determining the experiment result.
3. All samples, washing concentration and wastes of every kind should be disposed as infective agent.
4. Other reagents not needed must be packed or covered. Reagents of different batches must not be mixed and should be used before their respective validity dates.
5. Add Sample: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended
6. Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature

7. Reaction Time Control: Please control reaction time strictly following this product description!

8. Stop Solution: As it is an acid solution, please pay attention to the protection of your eyes, hands, face and clothes when using this solution.

9. Mixing: You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the ELISA plate frame gently with your finger before reaction.

10. Security: Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.

11. Do not use components from different batches of kit (washing buffer and stop solution can be an exception).

12. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. Otherwise, the results will be inaccurate!

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. It's recommended that all samples and standards be assayed in duplicate.

1. Secure the desired number of coated wells in the holder.

2. Dispense 10μL of standards, controls and specimens into appropriate wells.

3. Dispense 50μL Testosterone Biotin Conjugate into each well.

5. Dispense 50μL of Testosterone Enzyme Conjugate into all well.

6. Mix well and Incubate at room temperature (20-25°C) for 60 minutes.

7. Remove liquid from all wells. Wash wells five times with 300μL of 1X wash buffer. Blot on absorbance paper or paper towel.

8. Dispense 50μL of Substrate A and 50μL of Substrate B into each well. Incubate at room temperature (20-25°C) for 20 minutes.

9. Stop the reaction by adding 50μL of Stop Solution to each well.

10. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

11. Read absorbance at 450nm with a microplate reader within 15 minutes.

Calculation

Make concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration; point to point or calculate the linear regression equation of standard curve according to the concentration of the standards and the OD value. Then substitute with the OD value of the samples to calculate their concentration.

X= Mouse Testosterone Concentration

A typical standard curve of ZellBio GmbH **Mouse Testosterone** Assay kit
(The chart is indicative only)

Sensitivity:

The minimum detectable dose of Testosterone is 0.02ng/mL (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest analyte concentration that could be differentiated from zero).

Assay Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level **Mouse Testosterone** were tested 12 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level **Mouse Testosterone** were tested on 3 different plates, 10 replicates in each plate.

$$CV\ (\%) = (SD/mean) \times 100$$

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Detection Range:

0.08-16ng/mL

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix.
	Wells not completely aspirated	Completely aspirate wells between steps.
Low signal	Too brief incubation times	Ensure sufficient incubation time;
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	
Deep color but low value	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
		Open the Plate Reader ahead to pre-heat
Large CV	Inaccurate pipetting	Check pipettes
High Background	Concentration of detector too high	Use Recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions
	Stop solution not added	Stop solution should be added to each well before measurement

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