Goat Testo(Testosterone) ELISA Kit
Cat#: orb782870 (ELISA Manual)

For research use only. Not intended for diagnostic use.

Sensitivity: 17.7 pg/mL

Detection range: 62.5-4000 pg/mL

Specificity: This assay has high sensitivity and excellent specificity for detection of Goat Testo. No significant cross-reactivity or interference between Goat Testo and analogues was observed.

Please refer to the outer packaging label of the kit for the specific shelf life.

KIT components & storage

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C （6 months）
Standard (lyophilized)	1	2	4°C/-20°C （6 months）
Standard/Sample Diluent Buffer	10mL	20mL	4°C
Biotinylated-Conjugate (100x)	30μL	60μL	4°C/-20°C （6 months）
Biotinylated Conjugate Diluent	5mL	10mL	4°C
Streptavidin-HRP (100×)	60μL	120μL	4°C/-20°C （6 months）
HRP Diluent	6mL	12mL	4°C
Wash Buffer (25×)	10mL	20mL	4°C
TMB Substrate Solution	6mL	9mL	4°C（lucifuge）
Stop reagent	3mL	6mL	4°C
Plate Covers	1	2	4°C

Biorbyt Ltd.
5 Orwell Furlong, Cowley Road,Cambridge, Cambridgeshire
CB4 0WY, United Kingdom
Email: info@biorbyt.com | Phone: +44 (0)1223 859 353 | Fax: +44(0)1223 280 240

Biorbyt LLC
Suite 103, 369 Pine Street, San Francisco
California 94104, United States
Email: info@biorbyt.com | Phone: +1 (415)-906-5211 | Fax: +1 (415) 651 8558

Special Explanation

1.Please store the kit at 4° C if used up in one week.

2.If used for more than 1 week, store the Pre-coated Microplate, Standard, Biotinylated-Conjugate and Streptavidin-HRP at -20° C and all other reagents at 4° C according to the temperature indicated on the label.

3.Avoid repeated freezing and thawing.

4.Do not use the kit after the expiration date.

5.Please check whether all components are complete after opening the package.

All kit components have been formulated and quality control tested to function successfully as a kit. Do not mix or substitute reagents or materials from other kit, performance cannot be guaranteed if utilized separately or substituted.

Materials Required, Not Supplied

1.Microplate reader capable of measuring absorbance at 450 ± 10 nm.

2.High-speed centrifuge.

3.Electro-heating standing-temperature cultivator.

4.Absorbent paper.

5.Distilled or deionized water.

6.Single or multi-channel pipettes with high precision and disposable tips.

7.Precision pipettes to deliver 2 μL to 1mL volumes.

Safety notes

1.This kit is sold for lab research and development use only and not for use in humans or animals.

2.Reagents should be treated as hazardous substances and should be handled with care and disposed of properly.

3.Gloves, lab coat, and protective eyewear should always be worn,Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Test Principle

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with D-Dimer(D2D) protein. Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to D-Dimer(D2D). Next,Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of D-Dimer(D2D) in the samples is then determined by comparing the OD of the samples to the standard curve.

Sample collection and storage

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles.

Tissue homogenates -The preparation of tissue homogenates will vary depending upon tissue type.

1.Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.

2.Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein)(PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).

3.The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.

4.Then, the homogenates were centrifuged for 5 minutes at 10000×g. Collection the supernatant and assay immediately or aliquot and store at ≤-20°C.

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1.Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly).

2.Wash cells three times in cold PBS.

3.Cells were then resuspended in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.

4.Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Urine -Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤-20°C.Avoid

repeated freeze-thaw cycles.

Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C. Remove particulates and assay immediately or store samples in aliquot at ≤-20°C. Avoid repeated freeze/thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note

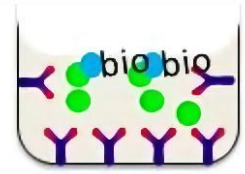
1.Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles.

2.Sample hemolysis will influence the result, so hemolytic specimen should not be used.

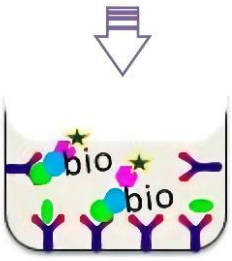
3.When performing the assay,bring samples to room temperature.

4.If the concentration of the test material in your sample is higher than that of the standard product, please make the appropriate multiple dilution according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio.

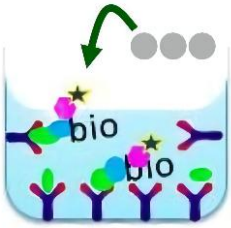
Summary



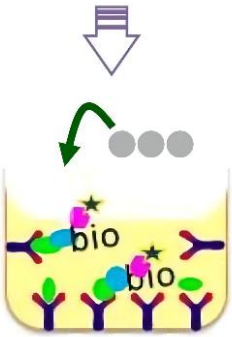
1. After the kit is equilibrated at room temperature, add 50µL of standard working solution or 50µL of sample to each well, immediately add 50µL of Biotinylated-antigen working solution to each well, mix well, incubate at 37°C for 60min .



2. Discard the liquid in the plate, add 200µL Wash Buffer to each well, and wash the plate 3 times. After drying, add 100µL Streptavdin-HRP working solution to each well, incubate at 37°C for 60min .



3. Discard the liquid in the plate, add 200µL Wash Buffer to each well, and wash the plate 5 times. After spin-drying, add 90µL TMB to each well, incubate at 37°C for 20min.



4. Add 50µL stop solution to each well, read plate at 450nm immediately, calculation of the results.

Reagent preparation

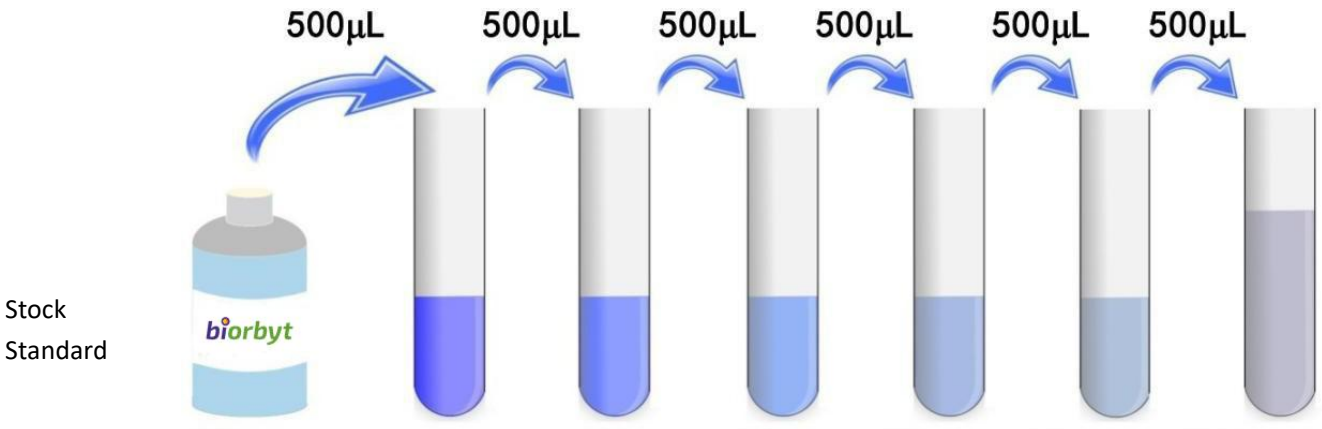
1.Bring all kit components and samples to room temperature (18-25°C) before use.

2.If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

3.Dilute the 25x wash buffer into 1x working concentration with double steaming water.

4.Biotinylated-Conjugate (1x) - Centrifuge the vial before opening. Biotinylated-Conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Biotinylated-Conjugate with 990 µL of Biotinylated-Conjugate Diluent.

5.**Standard** -Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is4000pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 4000pg/mL,2000pg/mL,1000pg/mL,500pg/mL,250pg/mL,125pg/mL,62.5pg/mL , and the last EP tubes with Standard Diluent is the blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.



6.Streptavidin-HRP (1x) - Centrifuge the vial before opening. Streptavidin-HRP requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Streptavidin-HRP with 990 µL of HRP Diluent.

7.**TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note

1.Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

2.Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

3.All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.

4.If crystals have formed in the Wash Solution concentrate (25×), warm to room temperature and mix gently until the crystals are completely dissolved.

5.Prepare standards within 15 minutes before assay. This standard can only be used once.

6.The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.

7.When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells. Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.

8.It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box.

Samples preparation

1.Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

2.The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Assay Procedure

1.Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

2.Prepare all reagents, working standards, and samples as directed in the previous sections.

3.Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2 - 8°C.

4.Set a Blank well with Standard Diluent Buffer. Add 50 µL of Standard or Sample to per well. Add 50 µL of Biotinylated -Conjugate(1x) to each well. Mix well, Cover with the adhesive films provided. Incubate for 1 hour at 37°C.

5.Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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 100 µL of Streptavidin-HRP (1x) to each well. Cover with the adhesive films provided. Incubate for 1 hour at 37°C.

7.Aspirate each well and wash, repeating the process for a total of five washes. Wash by filling each well with Wash Buffer (200 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. 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.

8.Add 90 µL of Substrate Solution to each well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.

9.Add 50 µL of Stop Solution to each well. When the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

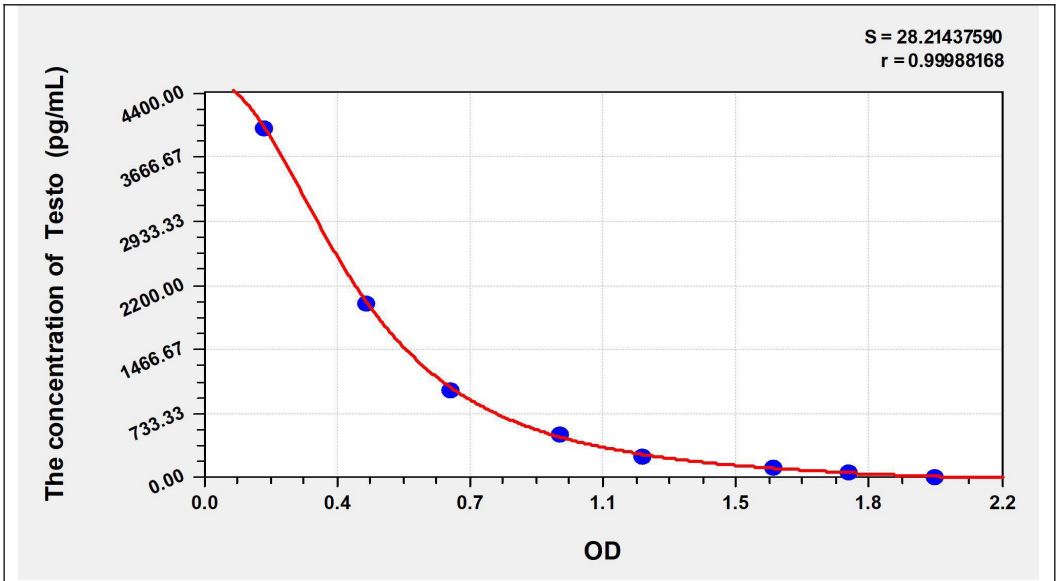
10.Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

11. *Samples may require dilution. See Sample Preparation section.

Calculation of Results

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between Goat Testo concentration in the sample and the assay signal intensity. Average the duplicate readings for each standard, control, and samples. Create a standard curve with the Goat Testoconcentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

Concentration pg/mL	OD
4000	0.183
2000	0.467
1000	0.696
500	0.998
250	1.225
125	1.585
62.5	1.789
0	2.028



Note: this graph is for reference only

Precision

Intra-assay Precision (Precision within an assay)：**CV%<8%**

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)：**CV%<10%**

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

Recovery

Matrices listed below were spiked with certain level of recombinant Testo and the recovery rates were calculated by comparing the measured value to the expected amount of Testo in samples.

Matrix	Recovery range	Average
serum(n=5)	90-105%	97%
EDTA plasma(n=5)	95-107%	101%
heparin plasma(n=5)	78-92%	89%

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Testo and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	85-96%	87-101%	95-103%	78-95%
EDTA plasma(n=5)	85-101%	78-96%	79-98%	93-102%
Heparin plasma(n=5)	82-101%	85-101%	87-96%	91-101%

Declaration

1.The kit may not be suitable for special experimental samples where the validity of the experiment,itself is uncertain, such as gene knockout experiments. 2.Certain natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins,may
not be detected because they do not match the detection antibody and capture antibody used in this product.
3.This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.

TROUBLESHOOTING GUIDE

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Color development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used	When TMB is used as the substrate, the
	when the absorbance value was read	absorbance should be read at 450 nm.

NO color plate

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read clearly when labels preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN3, etc.), and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN3
	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.

Description of results	Possible reason	Recommendations and precautions
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN3, etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.
	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.

Description of results	Possible reason	Recommendations and precautions
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The color of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual