Lablisa® ADT(Androsterone) ELISA Kit

Cat: LAB13359

For research use only. Not intended for diagnostic use.

Sensitivity: 144 pg/mL

Detection Range: 1406.25-90000 pg/mL

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

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

KIT Components

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C
Reference Standard (Lyophilized)	1 vial	2 vials	4°C/-20°C
Concentrated Biotinylated Detection Ab (100×)	60 µL	60 µL	4°C/-20°C
Concentrated HRP Conjugate (100×)	60 µL	120 µL	4°C/-20°C
Standard &Sample Diluent	20 mL	20 mL	4°C/-20°C
Biotinylated Detection Ab Diluent	13 mL	13 mL	4°C/-20°C
HRP Conjugate Diluent	13 mL	13 mL	4°C/-20°C
Concentrated Wash Buffer (25×)	30 mL	30 mL	4°C/-20°C
Substrate Reagent	10 mL	10 mL	4°C/-20°C (store in dark)
Stop Solution	10 mL	10 mL	4°C/-20°C
Plate Covers	5 pieces	5 pieces	RT

Special Explanation

1. *If the kit is opened, Store the whole kit at 4°C. If the kit is not used up in 1 week. Store the Pre-Coated Microplate, Standard ,Biotinylated Detection Ab and HRP Conjugate at -20°C, the rest reagents at 4°C, please used up within 6 months.
   *If the kit is not opened, store the whole kit: 4°C(short time storage, valid for 6 months); -20°C (long-term storage, valid for 1 year). Avoid repeated freeze-thaw cycles
2. Do not use the kit beyond the expiration date.
3. If the whole kit is stored at -20°C, place the kit at 4°C the day before the experiment.
4. After opening the package, please check that all components are complete.
5. The cap must be tightened to prevent evaporation and microbial contamination. The reagents volume is slightly more than the volume marked on labels, please use accurate measuring equipment and do not pour directly into the vial.

All kit components have been formulated and quality control tested to function successfully. Do not mixor substitute reagents or materials from other kits, detection effect of the kit will not 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. Double distilled water or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.
7. Precision pipettes to deliver 2 μL to 1 mL volumes.

Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Stop Solution and Substrate Reagent. In case of contact, wash thoroughly with water.

Test Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to ADT. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for ADT and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain ADT, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of ADT. You can calculate the concentration of ADT in the samples by comparing the OD of the samples to the standard curve.

Sample Collection and Storage

Serum - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000 × g for 20 minutes. 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 at 1000 × g and2-8°C for 15 minutes 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. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenize 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, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000 × g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

*Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

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

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000 × g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500 × g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

Urine - Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

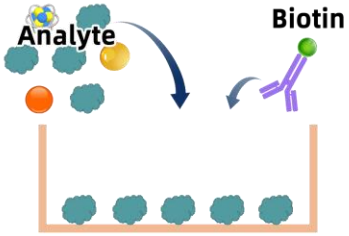
Saliva - Collect saliva using a collection device or equivalent. Centrifuge samples at 1000 × g at 2-8°C for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernatants and other biological fluids - Centrifuge samples at 1000 × g for 20 minutes. 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.

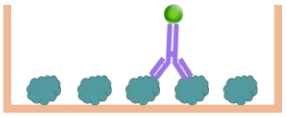
Notes

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 bio-activity and contamination. Avoid repeated freeze-thaw cycles.
2. Sample hemolysis will influence the result, so it 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 dilutions according to the actual situation (it is recommendedto do preliminary experiment to determine the dilution ratio.

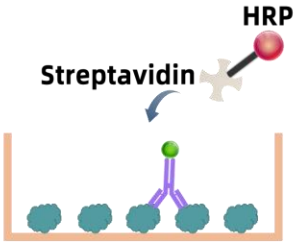
Summary



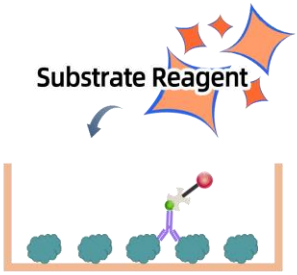
1、Add 50μL standard or sample to the wells, immediately add 50μL Biotinylated Detection Ab working solution to each well. Incubate for 30 min at 37℃



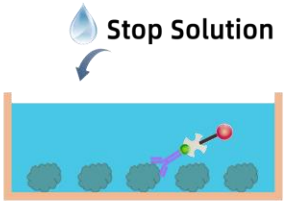
2、Aspirate and wash the plate for 3 times.



3、Add 100μL HRP conjugate working solution. Incubate for 30 min at 37℃. Aspire and wash the plate for 3 times



4、Add 100μL Substrate Reagent. Incubate for 15 min at 37℃



5、Add 50μL Stop Solution

6、Read the plate at 450nm immediately. Calculation of the results

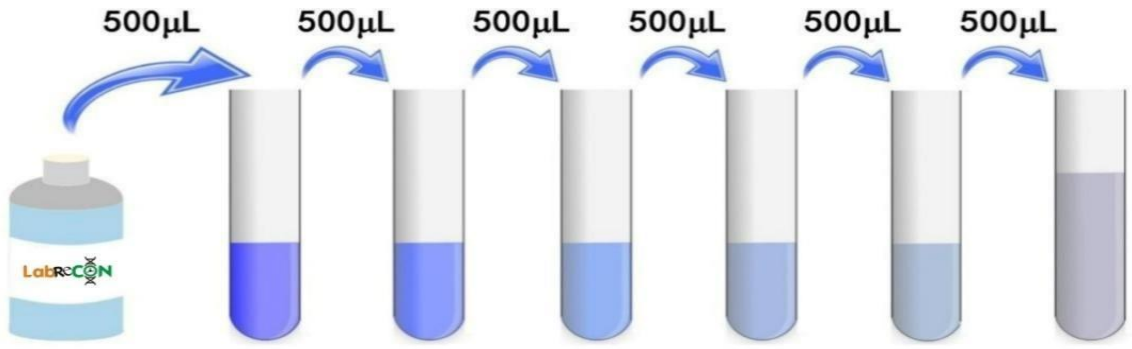
Reagent Preparation

1.Bring all kit components and samples to room temperature (18-25°C) before use.Make sure all components are dissolved and mixed well before using the kit.

2.If the kit will not be used up in 1 time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.

3.Dilute the 25× Wash Buffer into 1× Wash Buffer with double distilled water.

4.**Standard Working Solution -** Centrifuge the Standard at 1000 × g for 1 minute. Reconstitute the Standard with 1.0 mL of Standard Diluent Buffer, kept for about 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 90000 pg/mL. Please prepare 7 tubes containing 0.5 mL Standard Diluent Buffer and use the Diluted Standard to produce a double dilution series according to the picture shown below. To mix each tube thoroughlybefore the next transfer, pipette the solution up and down several times. Set up 7 points of Diluted Standard such as 90000 pg/mL, 45000pg/mL, 22500pg/mL, 11250 pg/mL,5625pg/mL, 2812.5pg/mL, 1406.25 pg/mL, and the last EP tubes with Standard Diluent is the Blank as 0pg/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high concentration to low concentration, replace the pipette tip for eachdilution. Note: the last tube is regarded as the Blank and do not pipette solution into it from the former tube.



90000	45000	22500	11250	5625	2812.5	1406.25
pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL

5.**1× Biotinylated Detection Ab and 1× HRP Conjugate Working Solution -** Briefly spin or centrifuge the stock Biotinylated Detection Ab and HRP Conjugate before use. Dilute them to the working concentration 100 folds with Biotinylated Detection Ab Diluent and HRP Diluent, respectively. For example, 10 µL of HRP Conjugate with 990 µL of HRP Diluent.

6.**Substrate Reagent** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Notes

1. After receive the kit, please store the reagents according to the instructions. The plates can be disassembled to single strips. Please use it in batches on demands.
2. The test tubes, pipette tips and reagents used in the experiment are all disposable and are strictly prohibited from being reused; otherwise the experiment results will be affected. Kit reagents of different batches cannot be mixed (except Substrate Reagent, Washing Buffer and Stop Solution).
3. Lyophilized Standards, Biotinylated Detection Ab , and HRP Conjugate are small in volume and may bescattered in various parts of the tube during transportation. Please centrifuge at 1000 × g for 1 minute before use. Then, carefully pipette 4-5 times to mix the Solution. Please configure the Standard, 1× Biotinylated Detection Ab and 1× HRP Conjugate Working Solution according to the required amount, and use the corresponding Dilution Solution, cannot be mixed used.
4. Bring all reagents to room temperature (18-25°C) before use. If crystals form in the concentrate (25×), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C), gently Mix until crystals are completely dissolved.
5. Prepare to dissolve Standard within 15 minutes before the test. This Standard Working Solution can only be used once. If the dissolved Standard is not used up, please discard it. The sample addition needs to be rapid. Each sample addition should preferably be controlled within 10 minutes. To ensure experimental accuracy, it is recommended to test duplicate wells, and when pipetting reagents, keep a consistent order of additions from 1 well to another, this will ensure the same incubation time for all wells.
6. During the washing process, the residual washing liquid in the reaction well should be patted dry on absorbent paper. Do not put the paper directly into the reaction well to absorb water. Before reading, pay attention to remove the residual liquid and fingerprints at the bottom, so as not to affect the microplate reader reading.
7. Substrate Reagent is light-sensitive, avoid prolonged exposure to light. Dispense the Substrate Reagent within 15 minutes following the washing of the microtiter plate. In addition, avoid contact between Substrate Reagent and metal to prevent color development. Substrate Reagent is contaminated if it turns blue color before use and should be discarded. Substrate Reagent is toxic, avoid direct contact with hands.

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

Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mixall 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.
3. 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. The Micro ELISA Plate slats to be used were removed from the plate frame and the remaining slats were returned to the aluminum foil bag containing the desiccants and then resealed for storage.

2. Determine wells for diluted standard, blank and sample. Add 50µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Immediately add 50µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.

3. Decant the solution from each well add 300µL of wash buffer to each well. Soak for 0.5 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.

4. Add 100µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

5. Decant the solution from each well, repeat the wash process for 3 times as conducted in step 3.

6. Add 100µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before

OD measurement.

7. Add 50µL of Stop Solution to each well. Note: adding the stop solution should be done in the same order as the substrate solution.

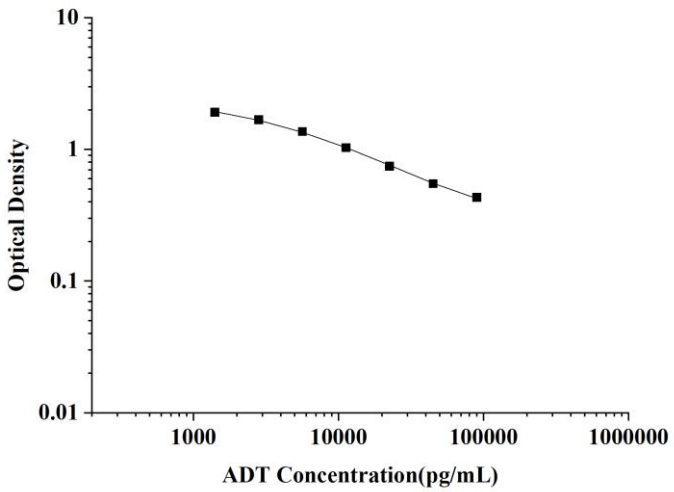
8. Determine the optical density (OD value) of each well at once with a micro plate reader set to 450 nm.

Note: Samples may require dilution (please refer to Sample Preparation section).

Calculation of Results

1. Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Plot a four parameters logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

2. If the OD of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor..

pg/mL	OD	Standard Curve
90000	0.434	
45000	0.55	
22500	0.746	
11250	1.031	
5625	1.369	
2812.5	1.684	
1406.25	1.92	
0	2.439	

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-assayprecision.

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 ADT and the recovery rateswere calculated by comparing the measured value to the expected amount of ADT in samples.

Sample Type	Range(%)	Average Recovery(%)
Serum(n=8)	87-95	91
EDTA Plasma(n=8)	81-95	88
Cell culture media(n=8)	83-97	90

Linearity

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

		Serum (n=8)	EDTA Plasma (n=8)	Cell culture media (n=8)
1:2	Range(%)	85-94	85-103	87-98
	Average(%)	87	101	91
1:4	Range(%)	90-99	85-103	80-92
	Average(%)	92	87	85
1:8	Range(%)	82-101	83-96	96-105
	Average(%)	82	94	104
1:16	Range(%)	91-102	95-102	86-97
	Average(%)	96	97	92

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.

Analysis of Common Problems and Causes of ELISA Experiment

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 washedsufficiently	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
	HRP Conjugate contaminates the tip and Substrate Reagent container or positive control contaminates the Pre-coatedMicroplate	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 Detection Ab or HRP Conjugate 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 the absorbance value was read	When Substrate Reagent is used as the substrate, the 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 plateare colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when 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 NaN_3, 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 NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3
	The sample to be tested may The standard is normal, the color of the sample is lightnot 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 Substrate Reagent is used as the substrate, theabsorbance 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 NaN_3, 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 wellof 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

STATEMENT: This manual is for your reference only; specifications are subject to the delivery manuals with the original product.