Canine TCC C5b-9 (Terminal Complement Complex C5b-9) ELISA Kit

Cat #: orb2938831 (manual)

For research use only. Not intended for diagnostic use

Product Features

Application	In vitro quantitative determination of TCC C5b-9 concentrations in serum, plasma, cell culture supernatant and other biological samples.		
Reactivity	Canine	Detection Method	Sandwich
Range	7.813-500ng/ml	Sensitivity	4.688ng/ml
Detection Duration	4 hours (excluding balancing and sample preparation)		
Samples needed for single well (Max)	Serum: 50ul, Plasma: 50ul, Cell Culture Supernatant: 100ul, cell or tissue lysate: 100ul, Other liquid samples: 50ul		
Specificity	Specifically recognize TCC C5b-9, no obvious cross reaction with other analogues		
Storage	2-8°C (for sealed box), please do not freeze!		

Background

TCC C5b-9

Terminal Complement Complex C5b-9 (TCC C5b-9), also known as the membrane attack complex (MAC), is an end-effect product of the activation of the complement system, consisting of complement components C5b, C6, C7, C8, and multiple C9 molecules. This complex plays a key role in pathogen clearance and immune defence by mediating cellular osmotic dissolution by forming pore structures on the target cell membrane. In addition to the cytolytic effect, the concentration of C5b-9 in lysate cells can also activate intracellular signalling pathways and participate in pathophysiological processes such as inflammation, cell proliferation and tissue injury. Abnormal deposits of C5b-9 are associated with a variety of diseases, including paroxysmal sleep hemoglobinuria (PNH), age-related macular degeneration (AMD), and ischemia-reperfusion injury. C5b-9 production depends on the triggering of classical, lectin, or bypass complement activation pathways and is commonly seen at sites of infection, autoimmune disease, or tissue injury.

Internal Test Data

Sample Type	Recommended Dilution Ratio	Content
Healthy serum	1/2 dilution	ND-15ng/ml
Healthy plasma	1/2 dilution	ND-20ng/ml

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Assay Principle

This kit is based on sandwich enzyme-linked immunosorbent assay (ELISA) technology. An anti-TCC C5b-9 capture antibody is pre-coated onto a 96-well microplate. A biotin-conjugated anti-TCC C5b-9 antibody is used as the detection antibody. Standards and samples are added to the wells and incubated to allow TCC C5b-9 to bind to the capture antibody. After incubation, unbound materials are removed by washing. The biotinylated detection antibody is then added to form a capture antibody–antigen–detection antibody "sandwich." After washing, HRP-streptavidin is added, followed by additional washing to remove unbound conjugate. TMB substrate is then added to visualize the HRP enzymatic reaction. HRP catalyzes the conversion of TMB to a blue-colored product, which turns yellow after the stop solution is added. Absorbance is read at 450 nm using a microplate reader. TCC C5b-9 concentration is determined by generating a standard curve; the concentration is proportional to the OD450 value.

Kit Components

An unopened kit can be stored at 2-8℃ for 12 months. If the opened kit is not used up, store the items separately according to the following conditions.

Item	Size(48T)	Size(96T)	Storage Condition for Opened Kit
ELISA Microplate (Dismountable)	8×6	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 12 months at -20°C
Lyophilized Standard	1vial	2vials	Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 12 months at -20°C
Biotin-labeled Antibody (Concentrated, 100X)	60ul	120ul	2-8°C (Avoid Direct Light)
HRP-Streptavidin Conjugate (SABC, 100X)	60ul	120ul	
TMB Substrate	5ml	10ml	
Sample Dilution Buffer	10ml	20ml	2-8°C
Antibody Dilution Buffer	5ml	10ml	
SABC Dilution Buffer	5ml	10ml	
Stop Solution	5ml	10ml	
Wash Buffer(25X)	15ml	30ml	
Plate Sealer	3 pieces	5 pieces	
Product Description	1 copy	1 copy	

Note: Liquid reagent bottles may contain slightly more volume than indicated on the label. Use a calibrated pipette for accurate measurement and dilution.

Materials Required but Not Supplied

- Microplate reader (450 nm)
- 37°C incubator (CO_2 incubators used for cell culture are not recommended)
- Automated plate washer or multichannel pipette/5 mL pipettor (for manual washing)
- Calibrated single-channel pipettes (0.5–10 µL, 5–50 µL, 20–200 µL, 200–1000 µL) and a multichannel pipette, with disposable tips
- Sterile tubes and microcentrifuge tubes
- Absorbent paper and a plate holder/tray
- Deionized or distilled water

Sample Preparation

Sample Collection and Storage

1. Serum: Allow whole blood to clot at room temperature for 2 hours or at 2–8°C overnight. Centrifuge for 20 minutes at 1,000 × g and collect the supernatant for immediate analysis. Alternatively, aliquot and store at −20°C or −80°C for future assays.

2. Plasma: EDTA-Na2/K2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2–8°C within 30 minutes after collection. Collect the supernatant for immediate analysis. Or you can aliquot the supernatant and store it at -20°C or -80°C for future assays. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Samples: Tissue samples should be prepared as homogenates as follows:

3.1 Place the tissue on ice. Remove residual blood by washing with pre-cooled PBS (0.01 M, pH 7.4), then weigh the tissue.

3.2 Homogenize on ice using lysis buffer. The required volume depends on tissue weight; typically, **9 mL PBS per 1 g tissue** is appropriate. Protease inhibitors are recommended (e.g., **1 mM PMSF**).

3.3 Further process the homogenate using sonication or freeze–thaw cycles (keep samples cold during sonication; freeze–thaw cycles may be repeated twice).

3.4 Centrifuge for **5 minutes at 5,000 × g**. Collect the supernatant for immediate analysis or aliquot and store at **−20°C or −80°C**.

3.5 Measure total protein concentration using a BCA assay for data normalization. Total protein concentration for ELISA is typically **1–3 mg/mL**. Some tissues (e.g., liver, kidney, pancreas) may contain high endogenous peroxidase activity that can react with TMB and cause false-positive results. If needed, inactivate with **1% H_2O_2 for 15 minutes**, then repeat the assay.

Notes: PBS or mild RIPA buffer may be used. If you use RIPA buffer, adjust to **pH 7.3**. Avoid NP-40, Triton X-100, and DTT, as these may significantly inhibit assay performance. A recommended lysis buffer is **50 mM Tris, 0.9% NaCl, 0.1% SDS, pH 7.3**. You may prepare this buffer in-house or contact Biorbyt for purchasing options.

4. Cell Culture Supernatant: Centrifuge at **2,500 rpm** at **2–8°C for 5 minutes**, then collect the clarified supernatant for immediate analysis. Alternatively, aliquot and store at **-80°C** for future assays.

5. Cell Lysate

5.1 Suspension Cells

Centrifuge at **2,500 rpm** at **2–8°C for 5 minutes** and collect the cells. Add pre-cooled PBS and mix gently, then centrifuge again to pellet the cells. Add **0.5–1 mL** lysis buffer with protease inhibitor (e.g., PMSF, final concentration **1 mmol/L**). Lyse on ice for **30–60 minutes** or disrupt by sonication.

5.2 Adherent Cells

Remove the supernatant and wash the cells three times with pre-cooled PBS. Add **0.5–1 mL** lysis buffer with protease inhibitor (e.g., PMSF, final concentration **1 mmol/L**). Scrape cells using a cell scraper. Lyse the suspension on ice for **30–60 minutes** or disrupt by sonication.

5.3 During lysis, mix by pipetting or gently shaking the tube to ensure complete lysis. Viscosity is typically caused by genomic DNA and can be reduced by sonication on ice (3–5 mm probe, 150–300 W, 3–5 seconds per pulse, 30-second intervals; total sonication time 1–2 minutes).

5.4 After lysis/sonication, centrifuge at **10,000 rpm** at **2–8°C for 10 minutes**. Transfer the supernatant to a microcentrifuge tube for immediate analysis, or aliquot and store at **−80°C**.

Notes: Refer to the tissue sample notes above.

6. Other Biological Sample

Centrifuge samples for **15 minutes at 1,000 × g** at **2–8°C**. Collect the supernatant for immediate analysis, or aliquot and store at **−80°C** for future assays.

Recommended reagents for sample preparation: 100mM PMSF protease inhibitor, Lysis Buffer (for ELISA).

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Recommended Sample Dilution Ratio

For guidance on sample dilution and background information, refer to the shipped instructions or contact Biorbyt.

Sample Type	Recommended Dilution Ratio	Content
Healthy serum	1/2 dilution	ND-15ng/ml
Healthy plasma	1/2 dilution	ND-20ng/ml

Important: Matrix components in serum/plasma can affect assay performance. Serum/plasma samples should be diluted at least **1:2** with Sample Dilution Buffer before testing.

If a different dilution ratio is required, refer to the universal dilution guide below. Volumes are for a single well, multiply by the number of wells for duplicates.

- **1:2 dilution:** Add 60 µL sample + 60 µL diluent; mix gently.
- **1:5 dilution:** Add 24 µL sample + 96 µL diluent; mix gently.
- **1:10 dilution:** Add 12 µL sample + 108 µL diluent; mix gently.
- **1:20 dilution:** Add 6 µL sample + 114 µL diluent; mix gently.
- **1:50 dilution:** Add 3 µL sample + 47 µL 0.9% NaCl into 100 µL diluent; mix gently.
- **1:100 dilution:** Add 3 µL sample + 177 µL 0.9% NaCl into 120 µL diluent; mix gently.
- **1:1,000 dilution:** Two-step dilution: prepare a 1:50 dilution first (normal saline), then prepare a 1:20 dilution; mix gently.
- **1:10,000 dilution:** Two-step dilution: prepare a 1:100 dilution first (normal saline), then repeat; mix gently.
- **1:100,000 dilution:** Three-step dilution: prepare 1:50 and 1:20 dilutions (normal saline for the first two steps), then prepare a 1:100 dilution; mix gently.

Note: Use at least **3 µL** sample in each dilution step. Mix gently to avoid foaming.

Notes: The volume in each dilution is not less than 3µL. Dilution factor should be within 100 fold. Mixing during dilution is required to avoid foaming.

Notes for Sample

1. Use disposable, endotoxin-free blood collection tubes. Avoid hemolyzed or lipemic samples.
2. Recommended storage: up to **5 days at 2–8°C**, up to **6 months at −20°C**, and up to **2 years at −80°C**. For longer storage, samples may be stored in liquid nitrogen.
3. Thaw frozen samples rapidly in a **15–25°C** water bath to minimize ice-crystal effects. After thawing, centrifuge to remove precipitates, then mix thoroughly.
4. The assay detection range does not necessarily match the native analyte concentration in all sample types. If concentration falls outside the range, dilute or concentrate on samples as needed.
5. A pilot test is recommended for uncommon sample types or models without reference data.

Recombinant proteins may not always be recognized by the kit antibodies, which may result in undetectable signal.

Reagent Preparation

Remove the kit from the refrigerator approximately **20 minutes** before use and equilibrate to room temperature (**18–25°C**). For repeated assays, remove only the strips and standards needed for the current run and store remaining components according to the recommended conditions.

1. Wash Buffer

Dilute **30 mL** (or **15 mL** for 48T) of concentrated wash buffer with deionized or distilled water to a final volume of **750 mL** (or **375 mL** for 48T) and mix thoroughly. (Recommended resistivity of ultrapure water: **18 MΩ**.) Alternatively, prepare a 25-fold dilution based on the volume required for your assay. Store unused diluted wash buffer at **2–8°C**.

If crystals are present in the concentrated wash buffer, warm in a **40°C** water bath until fully dissolved (do not exceed **50°C**). Use freshly prepared wash buffer the same day whenever possible. Any remaining diluted buffer should be used within **48 hours** when stored at **2–8°C**.

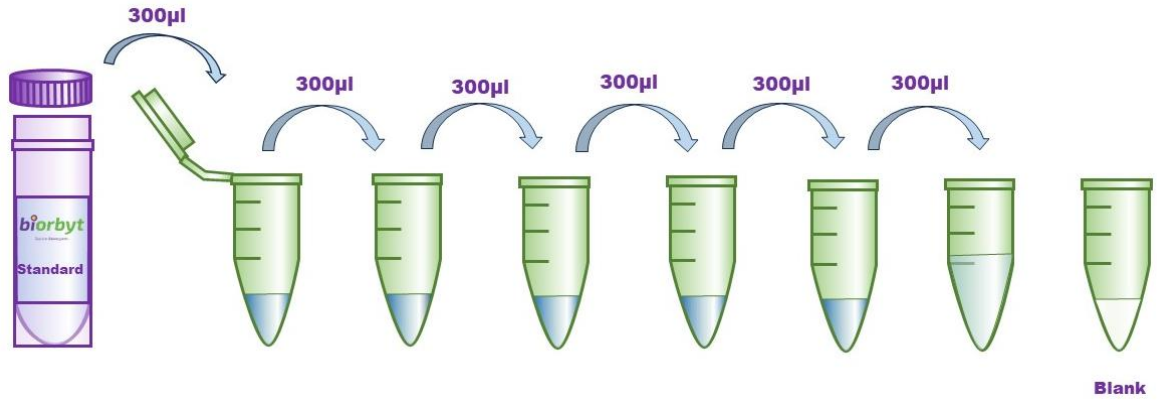
2. Standards

2.1 Centrifuge the standard tube for 1 minute at 10,000 × g and label as the Zero tube.

2.2 Add 1.0 mL sample dilution buffer to the standard tube. Cap tightly and allow to stand for 2 minutes at room temperature. Invert gently several times to mix (or vortex at low speed for 3–5 seconds).

2.3 Centrifuge for 1 minute at 1,000 × g to bring the liquid to the bottom of the tube and reduce bubbles.

2.4. Prepare the standard dilution series: Label 7 tubes as 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and blank. Add **0.3 mL** sample dilution buffer to each tube. Add **0.3 mL** from the Zero tube to the 1/2 tube and mix thoroughly. Transfer **0.3 mL** sequentially from tube to tube through 1/64, mixing thoroughly each time. The blank contains **0.3 mL** sample dilution buffer only. The standard concentrations from Zero to blank are: 500ng/ml, 250ng/ml, 125ng/ml, 62.5ng/ml, 31.25ng/ml, 15.625ng/ml, 7.813ng/ml, 0ng/ml.



Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

3. Preparation of Biotin-labeled Antibody Working Solution

Prepare this working solution within 30 minutes of use. Do not store for extended periods.

3.1 Calculate the required volume: 100 µL per well × number of wells (prepare an extra 100–200 µL).

3.2 Centrifuge the concentrated biotin-labeled antibody for 1 minute at 1,000 × g to collect liquid at the bottom of the tube.

3.3 Dilute the antibody 1:99 in antibody dilution buffer and mix thoroughly (e.g., 10 µL antibody + 990 µL dilution buffer).

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution

Prepare this working solution within 30 minutes of use. Do not store for extended periods.

4.1 Calculate the required volume: 100 µL per well × number of wells (prepare an extra 100–200 µL).

4.2 Centrifuge the concentrated SABC for 1 minute at 1,000 × g to collect liquid at the bottom of the tube.

4.3 Dilute the SABC 1:99 in SABC dilution buffer and mix thoroughly (e.g., 10 µL SABC + 990 µL dilution buffer).

Assay Procedure

Step 1: Add **100 µL** standard or sample to each well. Seal the plate and incubate for **90 minutes at 37°C**.
Washing: Wash the plate **twice** without soaking.

Step 2: Add **100 µL** biotin-labeled antibody working solution to each well. Seal the plate and incubate for **60 minutes at 37°C**.
Washing: Wash the plate **three times**, soaking for **1 minute** each time.

Step 3: Add **100 µL** SABC working solution to each well. Seal the plate and incubate for **30 minutes at 37°C**.
Washing: Wash the plate **five times**, soaking for **1 minute** each time.

Step 4: Add **90 µL** TMB substrate solution. Seal the plate and incubate for **10–20 minutes at 37°C** (monitor color development carefully).

Step 5: Add **50 µL** stop solution. Read absorbance at **450 nm** immediately and calculate results.

Detailed Assay Procedure

Mix all samples and reagents thoroughly during preparation. It is recommended to generate a standard curve for each assay.

1. Set up standard, pilot sample, and control (blank) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate to reduce experimental error.

2. Standards and sample loading: Aliquot 100 µL from the zero tube, 1st tube, 2nd tube, 3rd tube, and 4th tube into the corresponding standard wells. Add 100 µL of sample dilution buffer to the control (blank) well. Then add 100 µL of pilot samples to each sample well. Seal the plate and incubate statically at 37°C for 90 minutes. (Dispense solutions to the bottom of each well. Mix gently without touching the sidewalls or creating foam.)

3. Wash twice: Remove the plate sealer, aspirate the liquid, or tap the plate gently on clean absorbent paper two to three times. Add 350 µL of wash buffer to each well without immersion. Discard the liquid and tap the plate on absorbent paper again. Repeat this washing step twice.

4. Biotin-labeled antibody: Add 100 µL of biotin-labeled antibody working solution to each well. Seal the plate and incubate statically at 37°C for 60 minutes.

5. Wash three times: Remove the plate sealer, aspirate the liquid, or tap the plate gently on clean absorbent paper two to three times. Add 350 µL of wash buffer to each well and immerse for 1 minute. Discard the liquid and tap the plate on absorbent paper again. Repeat this washing step three times.

6. HRP-streptavidin conjugate (SABC): Add 100 µL of SABC working solution to each well. Seal the plate and incubate statically at 37°C for 30 minutes. (Place the entire bottle of TMB substrate in a 37°C incubator to equilibrate for 30 minutes.)

7. Wash five times: Remove the plate sealer and wash the plate five times with wash buffer, following the washing method described in step 5.

8. TMB substrate: Add 90 µL of TMB substrate to each well. Seal the plate and incubate statically at 37°C in the dark for 10–20 minutes. Turn on the microplate reader and allow it to preheat for 15 minutes.

(Notes: Do not use reagent reservoirs previously used for HRP-containing reagents. The reaction time may be shortened or extended based on color development but should not exceed 30 minutes. The reaction may be stopped when a clear gradient appears in the standard wells. Excessively weak or strong color development is unacceptable.)

9. Stop: Do not remove the liquid from the wells after color development. Add 50 µL of stop solution to each well. The color will immediately change from blue to yellow. Add the stop solution in the same order as the TMB substrate.

10. OD measurement: Measure absorbance immediately at 450 nm using a microplate reader. (If the reader supports wavelength correction, set a reference wavelength of 570 nm or 630 nm and subtract this value from the OD450 reading to correct for optical interference. If no reference wavelength is available, use the original OD450 value.)

Calculation of Results

1. Calculate the mean OD450 value (using either the original or corrected OD450 values) from the duplicate readings for each standard, control, and sample. Then obtain the calculated value by subtracting the OD450 blank.

2. Create a four-parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Exclude the OD450 blank during plotting.) Alternatively, curve-fitting software provided with the microplate reader may be used (e.g., Thermo SkanIt RE software or Curve Expert 1.3 or 1.4).

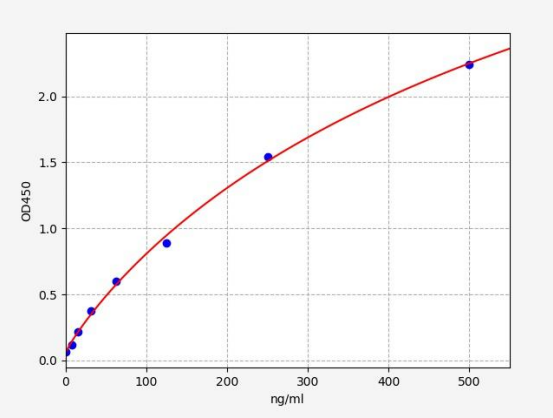
3. Determine the sample concentration by substituting the OD450 value into the standard curve. For diluted samples, multiply the calculated value by the corresponding dilution factor.

Typical Data & Standard Curve

This product has been tested by the Quality Control Department and meets the performance specifications described in this manual. (Laboratory humidity: 20%–60%; temperature: 18–25°C. TMB was equilibrated to 37°C before color development and incubated at 37°C for 15 minutes in the dark after addition.)

The following assay data are provided for reference only, as experimental conditions and technique may vary. Standard curve generation should be based on your assay conditions.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.065	0.067	0.066	0
7.813	0.113	0.117	0.115	0.049
15.625	0.214	0.22	0.217	0.151
31.25	0.373	0.383	0.378	0.312
62.5	0.588	0.606	0.597	0.531
125	0.875	0.901	0.888	0.822
250	1.519	1.563	1.541	1.475
500	2.21	2.274	2.242	2.176



Performance

Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Inter-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	15.18	61.76	227.6	15.99	59.78	234.3
Standard deviation	0.78	3.01	9.6	0.79	3.16	11.69
CV(%)	5.11	4.88	4.22	4.96	5.29	4.99

Recovery

Add a certain amount of TCC C5b-9 into the sample. Calculate the recovery by comparing the measured value with the expected amount of TCC C5b-9 in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-104	96
EDTA Plasma(n=5)	85-104	94
Heparin Plasma(n=5)	90-103	96

Linearity

Dilute the sample with a certain amount of TCC C5b-9 at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	85-101%	85-93%	87-95%
EDTA Plasma(n=5)	98-105%	84-95%	84-95%
Heparin Plasma(n=5)	92-102%	85-98%	82-97%

Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

Elisa kit(n=5)	37°C for 1 month	2-8°C for 6 months	2-8°C for 12 months
Average (%)	80	95-100	85-98

ELISA Troubleshooting

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 the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are	Mixed use of component reagents	Please read labels clearly when preparing or using

colorless; the positive control is not obvious	In the process of plate washing and sample enzyme contaminated addition, the marker is 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 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.
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.
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

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.

2. During the Elisa kit development, some endogenous interferons (not all) in the biological sample have been removed or decreased.

3. The final assay result is related to the validity of reagents, experimental operation and environment. Biorbyt is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.

4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.

5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.

6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter -assay CV between different batches may be caused by reasons before.

8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

9. This kit allows for research use only. For IVD or other purposes, Biorbyt is not responsible for relevant consequences and doesn't bear any legal liability.

Precautions for Kits

1. When using different Elisa kits, labelling is required to avoid mixed components and failed assay.
2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace.
3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.
4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.
5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.
6. Before confirmation, reagents from other batches or sources should not be used in this kit.
7. Don't reuse tips and tubes to avoid cross contamination.
8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.
9. Wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.