Rat GABA ELISA Kit

Cat #: orb567856 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Application	In vitro quantitative determination of GABA concentrations in serum, plasma, cell culture supernatant and other biological samples.		
Reactivity	Rat	Detection Method	Competitive
Range	31.25-2000pg/ml	Sensitivity	18.75pg/ml
Detection Duration	2 hours (excluding balancing and sample preparation)		
Samples needed for single well (Max)	Serum: 10ul, Plasma: 10ul, Cell Culture Supernatant: 50ul, cell or tissue lysate: 50ul, Other liquid samples: 50ul		
Specificity	Specifically recognize GABA, no obvious cross reaction with other analogues		
Storage	2-8°C (for sealed box), please do not freeze!		

Background

CAS: 56-12-2

GABA (γ-aminobutyric acid) is the primary inhibitory neurotransmitter in the central nervous system. It interacts with three receptor types: GABA(A), GABA(B), and GABA(C). The ionotropic GABA(A) and GABA(C) receptors are ligand-gated ion channels that mediate fast inhibitory synaptic transmission. In contrast, the metabotropic GABA(B) receptor is coupled to G proteins and modulates slow inhibitory synaptic transmission.

Internal Test Data

Sample Type	Recommended Dilution Ratio	Content
Healthy serum	1/5-1/10	0.3-4.6ng/ml
Rat brain tissue homogenates	undiluted	267pg/mg(total protein)

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Assay Principle

This kit is based on a competitive ELISA detection method. The microtiter plate is pre-coated with GABA. During the assay, GABA in the sample or standard competes with the immobilized GABA for binding sites on the biotinylated detection antibody specific to GABA. After incubation, excess conjugate and unbound sample or standard are removed by washing. HRP–streptavidin (SABC) is then added to each well and incubated, followed by the addition of TMB substrate solution. The enzymatic reaction is terminated by adding an acidic stop solution, and the color change is measured at 450 nm using a microplate reader. The concentration of GABA in the samples is determined by comparison with the standard curve and is inversely proportional to the OD450 value.

Kit Components

The sealed kit can be stored at 2-8 ℃. The storage condition for opened kit is specified in the table below:

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	2vial	Put the lyophilized Standard and Biotin-labeled Antibody (lyophilized) into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 12 months at -20°C
Biotin-labeled Antibody (Lyophilized)	1vial	1vial	
HRP-Streptavidin Conjugate (SABC, 100X)	60ul	120ul	2-8°C (Avoid Direct Light)
TMB Substrate	5ml	10ml	
Purified water	200ul	200ul	2-8°C
Sample Dilution Buffer	10ml	20ml	
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).

Recommended Sample Dilution Ratio

Please refer to the following table of recommended dilution ratio for limited samples for reference. (ND: Not Detected)

Sample Type	Recommended Dilution Ratio	Content
Healthy serum	1/5-1/10	0.3-4.6ng/ml
Rat brain tissue homogenates	undiluted	267pg/mg (total protein)

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 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.

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

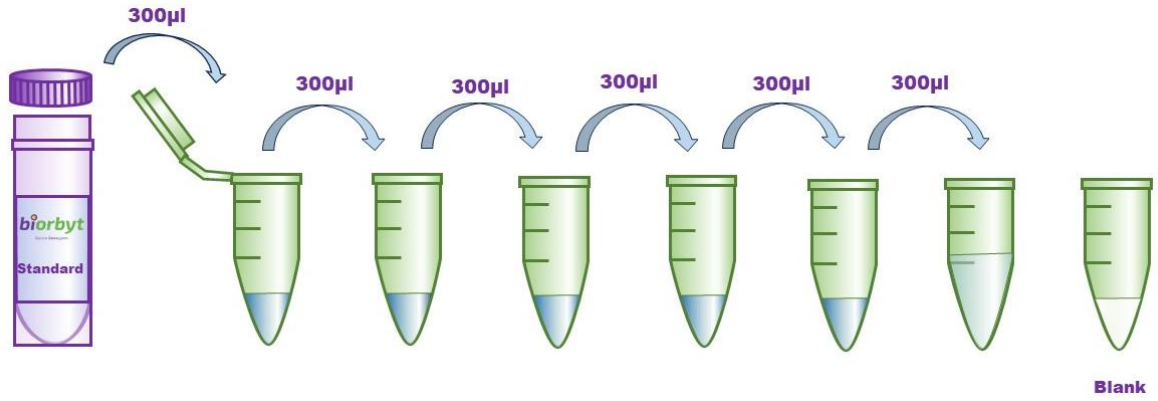
2.1 Centrifuge the standard tube for **1 minute at 10,000 × g** and label as the **Zero** tube.

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 Label seven tubes as 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and blank. Add 0.3 mL of sample dilution buffer to each tube. Add **0.3 mL** of the Standard to the 1/2 tube and mix thoroughly. Transfer **0.3 mL** from the 1/2 tube to the 1/4 tube and mix well. Then transfer **0.3 mL** from the 1/4 tube to the 1/8 tube and mix thoroughly. Continue this serial dilution stepwise through the 1/64 tube. The blank tube should contain only **0.3 mL** of sample dilution buffer. The standard concentrations from Zero to blank are: **2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 0pg/ml**.

Note: Store the reconstituted Zero tube at **2–8°C** and use within **12 hours**. Use diluted standard working solutions within **2 hours**.



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 Dissolution: Centrifuge the tube at 2,000 × g for 1 minute to collect the concentrated biotin-labeled antibody at the bottom. Add **70 µL** of purified water to the tube and mix thoroughly until fully dissolved. After reconstitution, store the biotin-labeled antibody at 2–8°C.

3.2 Calculate the total required volume of working solution as follows: **50 µL** per well × number of wells. It is recommended to prepare an additional 100–200 µL to ensure sufficient volume.

3.3 Dilute the biotinylated antibody **1:99** in antibody dilution buffer and mix thoroughly (e.g., 10 µL concentrated biotin-labeled antibody + 990 µL antibody 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.

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: Wash the plate **twice** before adding the Standard, Sample, and Control (blank) wells.

Step 2: Add **50 µL** of standard or sample to each well. Immediately add **50 µL** of biotin-labeled antibody to each well. Gently tap the plate for 1 minute to ensure thorough mixing, then incubate statically for 45 minutes at 37°C.
Washing: Wash the plate **three** times, allowing it to soak for 1 minute each time.

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

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

Step 5: Add **50 µL** of stop solution. Read the absorbance at 450 nm immediately and calculate the 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. Wash plate 2 times before adding standard, sample and control (blank) wells!
2. Aliquot 50 µL from the zero tube, 1st tube, 2nd tube, 3rd tube, and 4th tube into the appropriate standard wells. Add 50 µL of sample dilution buffer to the control (blank) well. Then, add 50 µL of pilot samples to each sample well. Immediately add 50 µL of biotin-labeled antibody working solution to each well. Gently tap the plate for 1 minute to ensure thorough mixing, then incubate statically for 45 minutes at 37°C. (Keep pipette tips or pipettors used for adding the biotin-labeled antibody away from the liquid surface.)
3. 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.
4. HRP-labeled Antibody: Add 100 µL of HRP-labeled antibody working solution to each well. Seal the plate and incubate statically at 37 °C for 30 minutes. (Place the entire bottle of TMB in a 37°C incubator to equilibrate.)
5. Wash five times: Remove the plate sealer and wash the plate five times with wash buffer, following the washing method described in step 3.
6. 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.)
7. 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.
8. 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).

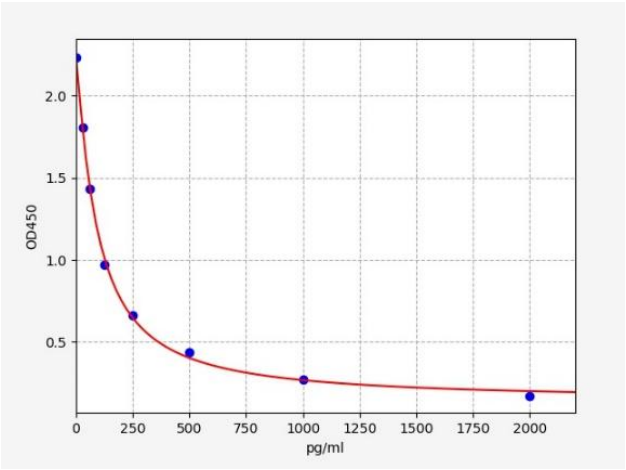
3. Determine the sample concentration by substituting the OD450 value into the standard curve. For diluted samples, multiply the calculated value by the corresponding dilution factor.

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.(pg/ml)	OD-1	OD-2	Average
0	2.2	2.264	2.232
31.25	1.78	1.832	1.806
62.5	1.414	1.456	1.435
125	0.956	0.984	0.97
250	0.655	0.673	0.664
500	0.434	0.446	0.44
1000	0.269	0.277	0.273
2000	0.169	0.173	0.171



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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/ml)	64.62	256.6	1025.2	62.49	250.6	1019.7
Standard deviation	3.49	9.62	38.96	2.32	13.53	38.03
CV(%)	5.4	3.75	3.8	3.71	5.4	3.73

Recovery

Add a certain amount of GABA into the sample. Calculate the recovery by comparing the measured value with the expected amount of GABA in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=10)	90-103	99
EDTA Plasma(n=10)	87-101	94
Heparin Plasma(n=10)	88-103	95

Linearity

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

Matrix	1:2	1:4	1:8
Serum(n=10)	86-104%	97-103%	89-102%
EDTA Plasma(n=10)	85-95%	83-100%	84-101%
Heparin Plasma(n=10)	82-97%	80-91%	84-99%

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 stopping the reaction, the entire plate shows a uniform yellow or light color, or the standard curve is linear but the background signal is excessively high.	Yellowing of the entire plate may be caused by incorrect addition of reagents.	Before starting the experiment, verify the components and lot numbers of all reagents to ensure they belong to the same kit. Reagents from different kits or different lot numbers must not be mixed.
	The ELISA plate was not washed sufficiently.	Ensure that the same volume of Wash Solution is added to each microwell during the washing process. After washing, firmly tap the ELISA plate on absorbent paper to remove any residual buffer.
	Incubation time was too long.	Strictly follow the procedures outlined in the manual.
	Streptavidin-HRP contaminated the pipette tip or TMB container, or the positive control contaminated the pre-coated microplate.	Replace pipette tips when dispensing different reagents. Use separate containers when preparing different reagent components, and always use a pipette during handling.
	The concentration of Biotinylated Antibody or Streptavidin-HRP was too high.	Verify that concentration calculations are correct, or perform further dilution if necessary.
	The substrate was exposed to light or contaminated prior to use.	Store reagents in the dark at all times prior to substrate addition.
	Color development time was too long.	Strictly follow the procedures outlined in the manual.
	An incorrect filter was used when reading the absorbance value.	When TMB is used as the substrate, measure absorbance 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	Components from different reagent sets were mixed.	Carefully read reagent labels when preparing or using them.

colorless, and the positive control is not clearly detectable. s	During plate washing or sample/enzyme addition, the enzyme label was contaminated or inactivated, resulting in loss of its ability to catalyze color development.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), and ensure that the container used to prepare the Wash Solution has been thoroughly cleaned.
	A reagent or procedural step was omitted.	Review the manual carefully and strictly follow the operating procedures.

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard appears normal, but the sample color is weak.	The sample contains NaN_3 preservative, which inhibits the enzyme reaction.	Samples must not contain NaN_3.
	The sample being tested may not contain strongly positive material, so the result may be normal.	If there is any doubt about the results, repeat the assay.
The visual result appears normal, but the microplate reader values are low.	An incorrect filter was used for absorbance measurement.	When TMB is used as the substrate, absorbance should be measured at 450 nm.
All wells, including Standards and Samples, show weak color development.	Insufficient incubation time.	Ensure accurate timing using a timer.
	Inadequate color development.	Typically 15–30 minutes.
	Excessive washing, or the dilution ratio of the concentrated Wash Buffer does not meet requirements.	Minimize the impact of washing by diluting the concentrated Wash Buffer and setting the washing time according to the manual. Accurately record the number of washes and the volume used.
	Poor quality distilled water.	The prepared Wash Buffer should be tested to confirm that the pH is neutral.
	During plate washing or sample addition, the enzyme label was contaminated or inactivated, resulting in loss of catalytic activity.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), that the container used to prepare the Wash Buffer has been thoroughly cleaned, and that the purified water used meets the required standards and is not contaminated.
	The kit has expired or was improperly stored.	Use the kit within its expiration date and store it according to the conditions recommended in the manual to avoid contamination.

	Reagents and samples were not equilibrated to room temperature before use.	Allow all reagents and samples to equilibrate at room temperature for approximately 30 minutes before use.
	Insufficient pipetting volume, overly rapid dispensing, excessive liquid remaining on the inner wall of the tip, or unclean tip walls.	Calibrate the pipette properly. Ensure tips are compatible and fit securely, pipette at an appropriate speed, and fully dispense the liquid. Tips should have clean inner walls and be used only once
Poor repeatability.	Incubation temperature was not properly controlled.	Maintain a constant incubation temperature and avoid localized temperature extremes.
	Excess liquid remained on the inner wall of the wells during liquid addition.	When adding liquids, dispense along the lower inner wall of the wells without touching the bottom.
	Reuse of consumables.	Replace pipette tips when drawing different reagents, and use separate containers when preparing different reagent components.
	The bottom of the microwell is scratched or contaminated.	Handle the plate carefully. Avoid touching the well bottoms and clean the bottom of the microplate to remove dirt or fingerprints.
		Perform technical replicates of the same sample three times, ensuring that at least two values are comparable.
	Cross-contamination during sample addition.	Minimize the risk of cross-contamination during sample addition.
The color development across the plate is uneven and irregular.	Cross-contamination during manual plate washing.	When washing plates manually, discard the first three washes immediately, then allow soaking during subsequent washes to reduce cross-contamination.
	Cross-contamination during plate tapping.	Use appropriate absorbent paper when tapping the plate. Avoid introducing foreign material into the wells, and avoid tapping in the same position repeatedly to reduce cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The color development of the plate is uneven and irregular	The liquid dispensing head of the plate washer is clogged, leading to improper liquid dispensing or excessive residual liquid after aspiration, which results in uneven and irregular color development across the plate.	Unclog the liquid dispensing head to ensure that each well is properly filled with wash solution during plate washing and that minimal residual liquid remains after aspiration.

	Incomplete centrifugation of the sample, resulting in coagulation within the reaction wells or interference from sediment or residual cellular components.	Serum and plasma samples should be fully centrifuged at 3000 rpm for more than 6 minutes.
	The sample was stored for an excessively long period, leading to contamination.	Samples should be kept fresh or stored at low temperatures to prevent contamination.
	Incorrect preparation of the Wash Solution or direct use of the concentrated Wash Solution.	Prepare all reagents strictly according to the manual.

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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.