TECHNICAL MANUAL

Bovine serum albumin (BSA) ELISA Kit

- **SKU CODE:** BOFI00128
- **SIZE:** 48T/96T
- **DETECTION PRINCIPLE:** Competitive (Antibody Coated)
- **RUO:** Research-Use-Only

Revised: 05/2026

Bovine serum albumin (BSA) ELISA Kit

Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.

Table of Contents

1. Key features

Detection method:

Competitive (Antibody Coated)

Sample Type:

For quantitative detection of BSA in serum, plasma, tissue homogenates and other biological fluids

Reactivity:

Bovine

Range:

0.156-10ug/ml

Sensitivity:

0.094ug/ml

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components as described in section 4. Date of expiration is on the ELISA Box label.

3. Product Description

The Assay Genie Bovine serum albumin (BSA) ELISA Kit is a highly sensitive assay for the quantitative measurement of BSA in the following samples: serum, plasma, tissue homogenates and other biological fluids.

This kit is based on the principle of a Competitive enzyme-linked immunosorbent assay (ELISA) principle, where the microtiter wells are pre-coated with an anti-bsa antibody. In this setup, the BSA present in the sample competes with the HRP-Antigen to bind the immobilized the antibody on the microtiter wells.

When the sample and the HRP-Antigen are added simultaneously, higher concentrations of BSA in the sample will block more coated antibody.

After washing away unbound materials, the TMB substrate is then added, producing a color reaction. The color intensity is inversely proportional to the concentration of BSA in the sample, higher sample concentrations result in a weaker color signal (lower OD at 450 nm).

The concentration of BSA is determined by comparing sample OD values to the standard curve.

This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.

4. Kit Contents

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below. Each kit contains reagents for either 48 or 96 assays including:

No	Component Name	Size 48T	Size 96T	Storage
1	ELISA Microplate (Dismountable)	8×6	8×12	2-8°C/-20°C
2	Lyophilized Standard	1vial	2vial	2-8°C/-20°C
3	Sample Dilution Buffer	10ml	20ml	20ml
4	HRP-labelled Antigen (Lyophilized)	1vial	1vial	2-8°C (Avoid Direct Light)
5	Purified Water	200ul	200ul	2-8°C
6	Antigen Dilution	5ml	10ml	2-8°C
7	TMB Substrate	5ml	10ml	2-8°C(Avoid Direct Light)
8	Stop Solution	5ml	10ml	2-8°C
9	Wash Buffer(25X)	15ml	30ml	2-8°C
10	Plate Sealer	3/5pieces		
11	Product Description	1copy		

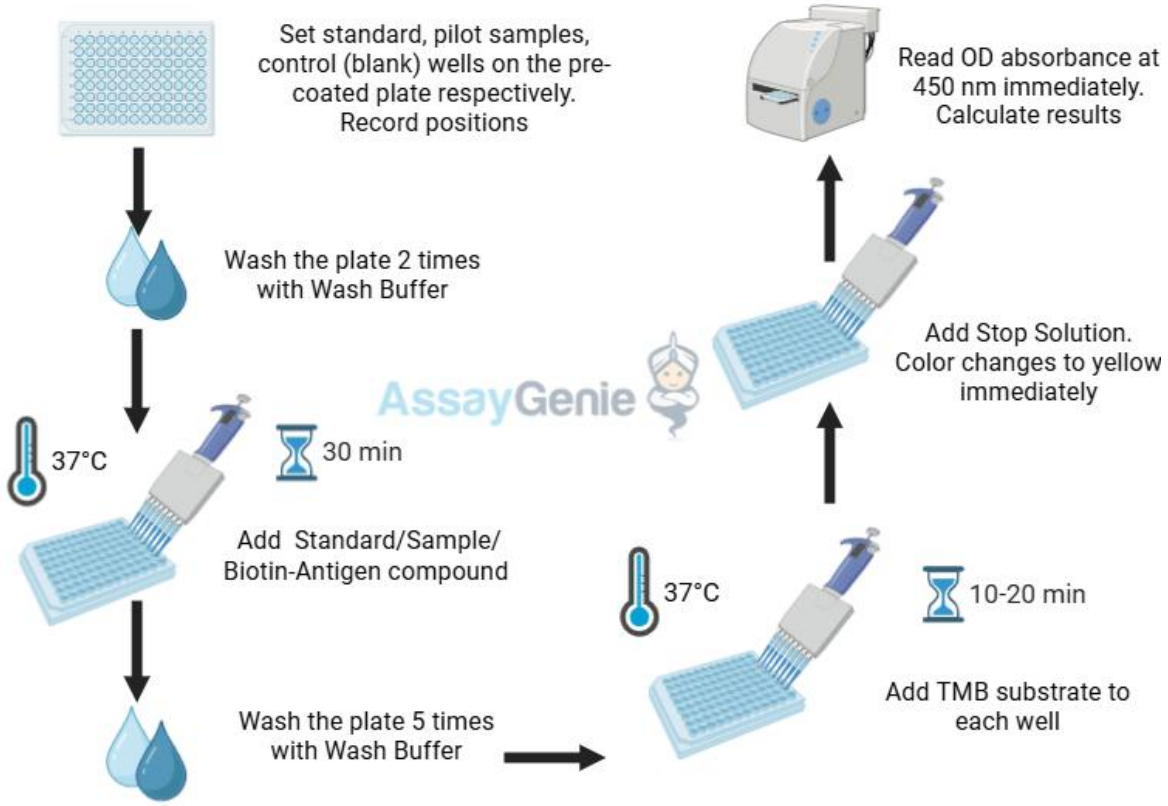
Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. This kit is for research purposes only and not for diagnostics or therapeutic uses.
2. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
3. Allow all reagents and samples to reach room temperature before use.
4. Ensure unopened and unused plates are kept dry to avoid contamination.
5. Before using the kit, centrifuge tubes to spin down standard and/or antibody.
6. Prepare all reagents, samples and standards as directed in this manual.
7. Duplicate wells are recommended for both standard and sample testing.
8. Do not let the microplate wells dry during the assay.
9. Maintain consistent incubation times and temperatures as variations can affect results.
10. Do not reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.

6. Assay Summary



7. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions**:
    - **Short-term**: 2-8 °C for up to 5 days.
    - **Medium-term**: -20 °C for up to 6 months.
    - **Long-term**: -80 °C or cryopreservation in liquid nitrogen.
- **Thawing Protocol**: Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles**: Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

A. Blood-Derived Samples

- **Serum:** Allow whole blood to coagulate at room temperature (2 h) or 2-8 °C overnight. Centrifuge at 1000 × g for 20 min and collect the supernatant. Store or use immediately.
- **Plasma:** Collect in anticoagulant tubes (EDTA, citrate, or heparin), mix gently, and centrifuge within 30 min at 1000 × g, 2-8 °C for 15 min. Store or assay as needed.

B. Tissue Homogenates

Tissue samples should be homogenized prior to use. Avoid buffers containing NP-40, Triton X-100, or DTT, as these strongly inhibit the assay. We recommend using 50 mM Tris + 0.9% NaCl + 0.1% SDS, pH 7.3.

The recommended protocol is as follows:

- **Sample Collection and Washing**
    - Place the target tissue on ice.

- Rinse the tissue with pre-cooled PBS buffer (0.01 M, pH 7.4) to remove residual blood.
- Weigh the tissue for further processing.

- **Homogenization**
  - Homogenize the tissue on ice using an appropriate lysis buffer.
  - The lysate volume should correspond to the tissue weight; typically, 9 mL PBS is used per 1 g of tissue. It is recommended to add protease inhibitors to the PBS (e.g., 1 mM PMSF). ***Note:*** *PBS buffer or mild RIPA lysis buffer can be used for homogenization. When using RIPA, adjust pH to 7.3.*
- **Cell Disruption**
  - Further disrupt the tissue using ultrasonic homogenization or freeze–thaw cycles.
    - Ultrasonic homogenization: Keep samples on an ice bath during sonication to avoid overheating.
    - Freeze–thaw cycles*:* Repeat twice for effective lysis.
- **Centrifugation and Storage**
  - Centrifuge the homogenate at 5000 × g for 5 minutes.
  - Collect the supernatant for immediate analysis, or aliquot and store at –20°C or –80°C for future assays.
- **Protein Concentration Measurement**
  - Determine total protein concentration using the Bradford Reagent included in this kit.
  - For ELISA assays, the total protein concentration should generally be 1–3 mg/mL
  - Tissues with high endogenous peroxidase levels (e.g., liver, kidney, pancreas) may react with TMB substrate, causing false positives. If this occurs, treat samples with 1% H_2O_2 for 15 minutes before repeating the assay.

Note: *Liver, kidney, and pancreas samples often contain high levels of endogenous peroxidase, which may react with the chromogenic substrate at elevated sample concentrations, potentially resulting in false positive signals.*

If analysis of these tissues is required, a gradient dilution assay is recommended. A proportional decrease in signal with increasing dilution typically indicates minimal interference and supports the accuracy of the results.

To further minimise potential interference, samples can be pre-treated with 1% hydrogen peroxide (H_2O_2) for 15 minutes prior to testing. To prepare the treatment solution, add 1 µl of pure H_2O_2 to 100 µl of sample (1% v/v).

C. Cell Culture Supernatant

Centrifuge the sample at 2500 rpm for 5 minutes at 2–8°C. Carefully collect the clarified cell culture supernatant for immediate analysis, or aliquot and store it at –80°C for future assays.

D. Cell Lysates

- **Suspension Cell Lysate:** Centrifuge the cell suspension at 2500 rpm for 5 minutes at 2–8°C and collect the cell pellet. Wash the pellet with pre-cooled PBS (0.01 M, pH 7.4) and mix gently. Repeat centrifugation and discard the supernatant. Add 0.5–1 mL of cell lysis buffer containing an appropriate protease inhibitor (e.g., PMSF, final concentration: 1 mM). Lyse the cells on ice for 30–60 minutes or disrupt them using ultrasonic homogenization.
- **Adherent Cell Lysate:** Remove the supernatant and wash the cells three times with pre-cooled PBS. Add 0.5–1 mL of cell lysis buffer supplemented with an appropriate protease inhibitor (e.g., PMSF at a final concentration of 1 mmol/L). Scrape the adherent cells using a cell scraper and transfer the cell suspension to a centrifuge tube. Lyse the cells on ice for 30–60 minutes, or disrupt the cells by ultrasonic treatment.

Follow next steps for protein extraction and supernatant collection:

- **Protein Release and DNA Disruption**
    - During lysis, pipette gently or intermittently shake the tube to enhance protein extraction.
    - Mucilaginous material formed during lysis is DNA, which can be broken down by ultrasonic disruption (3–5 mm probe, 150–300 W, 3–5 seconds per cycle, with 30-second intervals for 1–2 minutes total).
- **Supernatant collection**
    - After lysis or ultrasonic treatment, centrifuge the lysate at 10,000 rpm for 10 minutes at 2–8°C. Collect the supernatant for immediate use or aliquot and store at –80°C for future assays.

Notes: *Refer to the "Tissue Sample Notes" for additional buffer and inhibitor recommendations*.

E. Other Sample Types

For more information about how to process other sample types, (e.g., body fluids, breast milk & more), please contact our Tech Support Team at techsupport@assaygenie.com.

7.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit Bradford Protein Assay Protocol to view the full protocol.

8. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Note: *Set the height of the needles; be sure the fluid can be taken up completely.*

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: *Dilution may be necessary to minimize matrix effects. However, if the target concentration in the sample is very low, the pre-treated sample can be added directly to the assay without dilution.*

Reagent Preparation

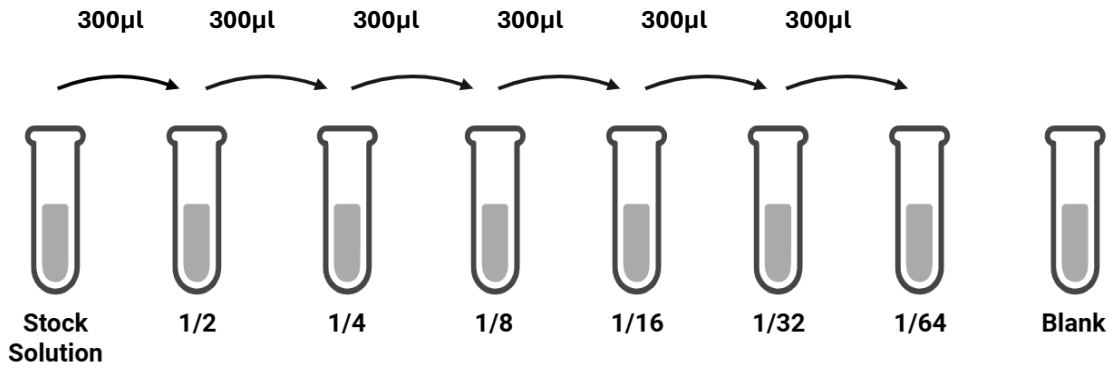
Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

A. Wash Buffer:

Dilute 30ml (15 ml for 48 well assay) of Concentrated Wash Buffer into 750 ml (375 ml for 48 well assay) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C for 48 hours. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

B. Standard Dilution:

1. Centrifuge standard tube for 1min at 10000xg.
2. Add 1 ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 2 min Invert the tube several times to mix gently. (Or you can mix it using a low-speed vortex mixer for 3-5 seconds.) ***Note:*** *If the standard vial concentration is different to the highest value in the range (please see page 3), please dilute using sample buffer to match highest range value to create stock solution.*
3. Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 300ul of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 300ul from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Note: *The standard solutions are best used within 2 hours. The standard solution series should be kept at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.*

C. Preparation of HRP-labelled Antigen working solution:

Prepare within 30 in before the experiment. This solution should not be stored for extended periods.

To ensure the performance of the test kit, the HRP-labelled antigen detection reagent should be stored at 2-8°C after reconstitution and used within 6 months. Prepare the HRP-labelled antigen working solution within 30 minutes before the experiment; it should be prepared fresh and is not suitable for long-term storage.

1. Dissolve: Centrifuge at 2000xg for 1 minute and collect the concentrated HRP-labelled antigen at the bottom of the tube. Add 70 µl of purified water to the HRP-labelled antigen tube, fully dissolve and mix well. Centrifuge for 1 min at 1000xg.
2. Calculate required total volume of the working solution: 55ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)
3. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated HRP-labelled antigen to the bottom of tube.

4. Dilute the HRP detection antigen with antigen dilution buffer at 1/100 and mix them thoroughly. (e.g. Add 10ul concentrated HRP-labelled antigen into 990ul antigen dilution buffer.)

D. Preparation Standard/Sample/HRP-Antigen compound:

Prepare within 30 minutes of starting the experiment.

1. When samples and standards are not tested repeatedly:

   - Take 55 µl prepared standard of each gradient and 55 µl HRP-labeled antigen working Solution and mix them well in EP tube for later use.
   - Take 55 µl diluted sample and 55 µl HRP-labeled antigen working Solution and mix well in EP tube for later use.

2. When the sample and standard are tested in duplicate:

   - Take 110 µl prepared standard of each gradient and 110 µl HRP-labeled antigen working Solution and mix them well in EP tube for later use.
   - Take 110 µl diluted sample and 110 µl HRP-labeled antigen working Solution and mix well in EP tube for later use.

9. Assay Procedure

1. **Reagent Preparation:** Equilibrate the TMB substrate at room temperature for at least 30 minutes before use. When diluting samples and reagents, ensure they are mixed thoroughly and evenly. It is recommended to prepare a standard curve for each assay.
2. **Plate Setup:** Designate standard wells, test sample wells, and control (blank) wells on the pre-coated plate, and record their positions. It is recommended to measure each standard and sample in duplicate. Wash the plate twice before adding standards, samples, and controls.
3. **Standard/Sample/HRP-Antigen Compound Loading:** Aliquot 100 µl of Standard/Sample/HRP-Antigen compound solution into each well and incubate for 30 minutes at 37°C.
4. **Washing:** Aspirate each well and wash five times with 350 µL of wash buffer. Allow the wash buffer to remain in the wells for 1–2 minutes during each wash. After the final wash, remove any residual wash buffer by aspiration or decanting..
5. **TMB Substrate:** Remove the cover and add 90 µL of TMB substrate solution to each well. Cover the plate and incubate at 37°C in the dark for 10–20 minutes. (***Note**: This incubation time is for reference only and should be optimised by the end-user*.) Once a blue colour develops in the first 3–4 wells (highest standards) and other wells show visible colour, proceed to the next step to terminate the reaction.
6. **Stop Solution:** Add 50 µL of Stop Solution to each well and mix thoroughly. The colour will change to yellow immediately.
7. **OD Measurement:** Read the optical density (OD) at 450 nm in a microplate reader immediately after adding the Stop Solution.

10. Data Analysis

This assay uses a competitive inhibition enzyme immunoassay format; therefore, the assay signal intensity is inversely proportional to the concentration of BSA in the sample.

Average the duplicate absorbance readings for each standard, control, and sample. Generate a standard curve by plotting BSA concentration on the y-axis against absorbance on the x-axis. Determine the best-fit line through the standard points using regression analysis.

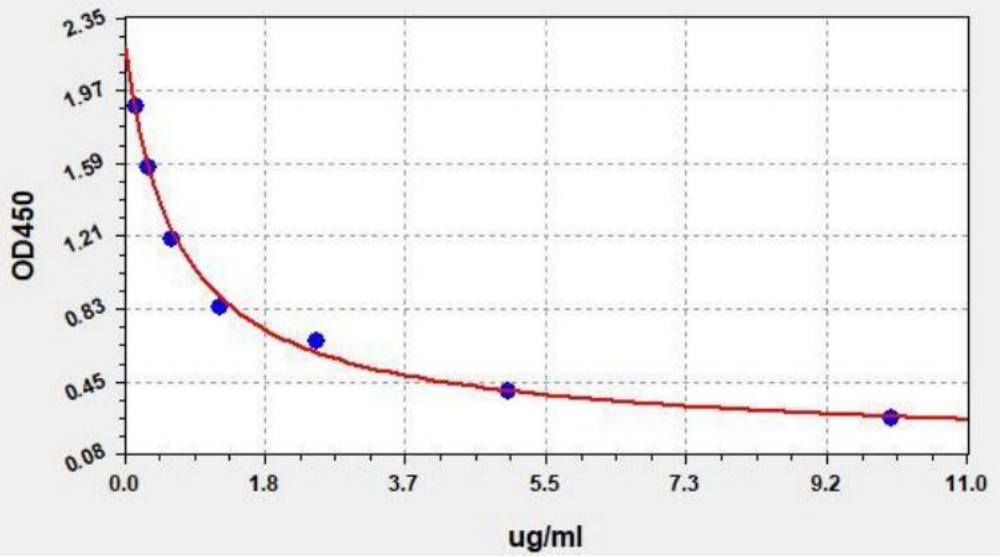
For diluted samples, multiply the concentration obtained from the standard curve by the corresponding dilution factor to calculate the final concentration. Curve fitting and data analysis may be performed using appropriate software (e.g., CurveExpert).

Note: *If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.*

11. Typical Data & Standard Curve

Standard Curve

Results of a typical standard run of an ELISA kit are shown below. This standard curve was



generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

Specificity

This assay has high sensitivity and excellent specificity for detection of Bovine serum No significant cross-reactivity or interference between Bovine serum albumin (BSA)and other molecules was observed.

Note: *Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Bovine serum albumin (BSA)and other molecules, therefore, cross reaction may still exist.*

Recovery

Matrices listed below were spiked with a certain level of Bovine serum and the recovery rates were calculated by comparing the measured value to the expected amount of Bovine serum albumin (BSA)in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-103	96
EDTA Plasma(n=5)	85-102	95
Heparin Plasma(n=5)	85-101	93

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Bovine serum albumin (BSA)and their serial dilutions.

Sample	1:2	1:4	1:8
Serum(n=5)	92-100%	88-104%	89-105%
EDTA Plasma(n=5)	86-99%	82-90%	87-100%

Heparin Plasma(n=5)	84-100%	87-101%	80-104%

Precision

- **Intra-assay Precision**: samples with low, medium and high concentration were tested 20 times on same plate.
- **Inter-assay Precision**: samples with low, medium and high concentration were tested 20 times on three different plates.

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of the Bovine serum albumin (BSA)ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

Standard (n=5)	**37°C for 1 month**	**4°C for 6 months**
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

12. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution.	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.