TECHNICAL MANUAL

Mouse NGB (Neuroglobin) ELISA Kit

- **SKU CODE:** AEKE05800
- **SIZE:** 96T/48T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 11/2025

Mouse NGB (Neuroglobin) 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.

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1. Key Features

Detection Method:

Sandwich

Sample Type:

Serum, Plasma, Tissue Homogenates and Other Biological Fluids.

Reactivity:

Mouse

Range:

0.32-20 ng/mL

Sensitivity:

0.136 ng/mL

2. Storage & Expiry

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

- 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-Conjugate and Streptavidin-HRP at -20°C, the rest of the reagents at 4°C, please used up within 6 months.
- If the kit is not opened, store the whole kit at 4°C (short time storage, valid for 6 months); -20°C (long-term storage, valid for 1 year). Avoid repeated freeze-thaw cycles.
- Do not use the kit beyond the expiration date.
- If the whole kit is stored at -20°C, place the kit at 4°C the day before the experiment.
- After opening the package, please check that all components are complete.

3. Product Description

The Assay Genie Mouse NGB (Neuroglobin) ELISA Kit is a highly sensitive assay for the quantitative measurement of a Mouse NGB in the following samples: Serum, Plasma, Tissue Homogenates and Other Biological Fluids..

This kit utilizes a sandwich enzyme-linked immunosorbent assay (ELISA) format. An antibody specific to the target protein is pre-coated onto the wells of a 96-well microplate. Samples and standards are added to the wells, allowing the target to bind to the immobilized antibody. After incubation, unbound substances are removed through washing.

A biotinylated detection antibody is then added, which binds specifically to the captured target. Following a second wash to remove excess detection antibody, HRP-conjugated Streptavidin is introduced, forming a biotin-streptavidin-HRP complex. After a third washing step, TMB substrate is added to initiate a colorimetric reaction catalysed by HRP. The reaction produces a blue product that turns yellow upon addition of the acidic Stop Solution.

The optical density (OD) is measured at 450 nm using a microplate reader. The OD450 value is directly proportional to the concentration of the target analyte in the sample, which can be determined by referencing a standard curve.

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

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays, please store the reagents per conditions below.

No	Component Name	Size 48T	Size 96T	Storage
1	Pre-Coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C
2	Standard (Lyophilized)	1 vial	2 vials	4°C/-20°C
3	Biotinylated Antibody (100×)	60 µL	120 µL	4°C/-20°C
4	Streptavidin-HRP (100×)	60 µL	120 µL	4°C/-20°C
5	Standard / Sample Diluent Buffer	10 mL	20 mL	4°C/-20°C
6	Biotinylated Antibody Diluent	6 mL	12 mL	4°C/-20°C
7	HRP Diluent	6 mL	12 mL	4°C/-20°C
8	Wash Buffer (25×)	10 mL	20 mL	4°C/-20°C
9	TMB Substrate Solution	6 mL	10 mL	4°C/-20°C (store in dark)
10	Stop Reagent	3 mL	6 mL	4°C/-20°C
11	Plate Covers	1 piece	2 pieces	RT
12	Bradford Reagent	1 vial	1 vial	4°C

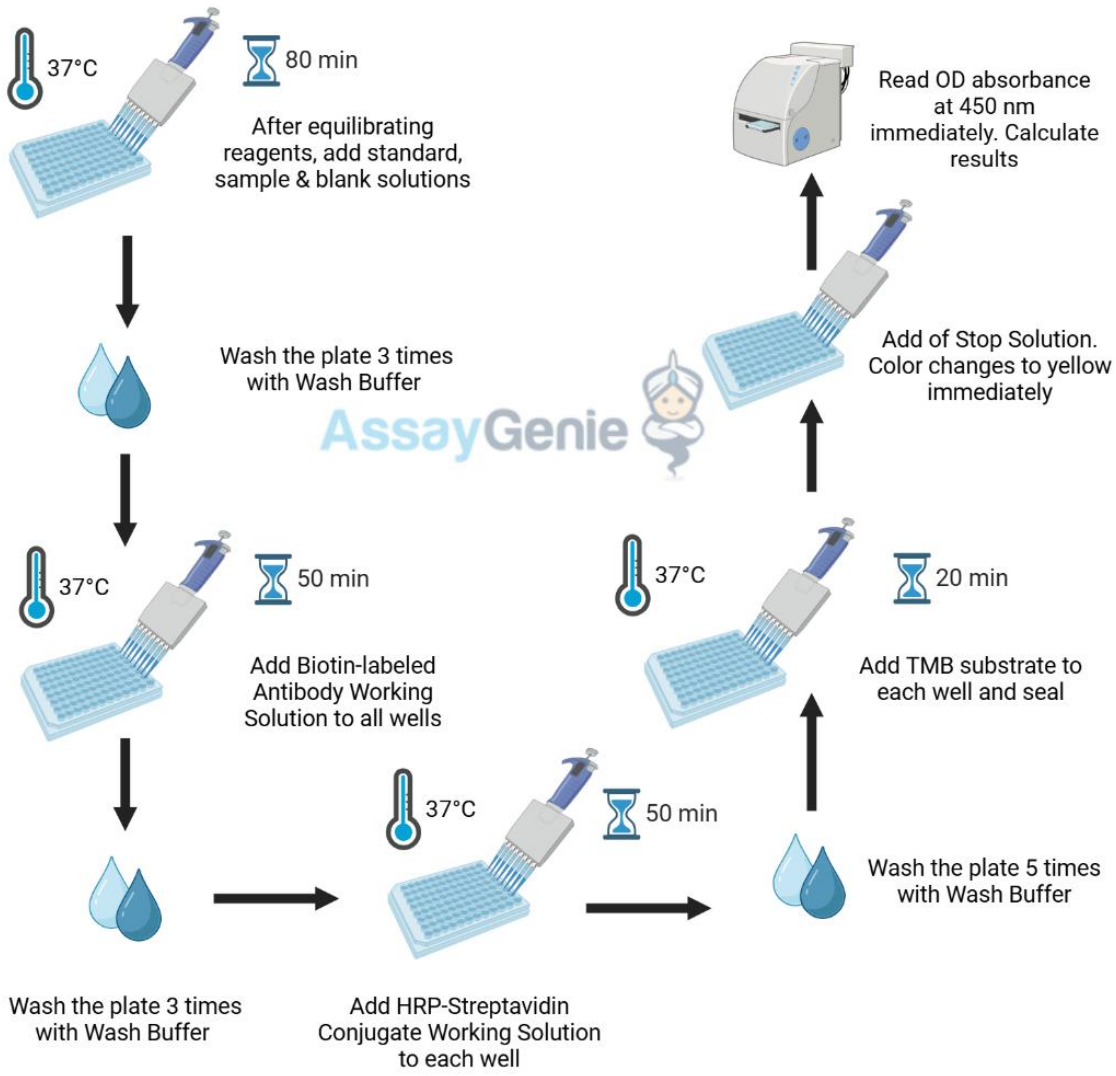
Additional materials required:

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.
7. High-speed centrifuge.

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 x 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 200 µl 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 200 µl 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.*

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 15 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures. ***Note:*** *If crystals form in the concentrate (25x), 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.*

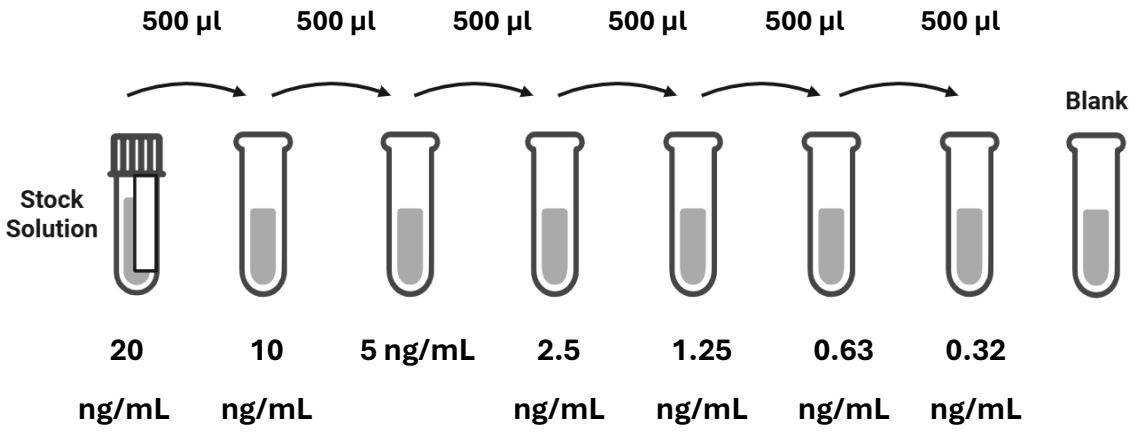
A. Wash Buffer Solution:

1. Dilute the 25x Wash Buffer into 1x Wash Buffer with double-distilled Water.

B. Standard Working Solution:

1. Centrifuge the Standard at 1000 x g for 1 minute. Reconstitute the Standard with 1 mL of Standard Diluent Buffer, kept for 10 minutes at room temperature, shake gently. ***Note:*** *Avoid the formation of foam.*
2. The concentration of the Standard Stock Solution is 20 ng/mL. Label 7 Eppendorf 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. Set up 7 points of Diluted Standard such as 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.32 ng/mL, and the last EP tubes with Standard Diluent is the Blank as 0 ng/mL. In order to guarantee the experimental results validity, please use the new Standard Solution for each experiment. When diluting the Standard from high

concentration to low concentration, replace the pipette tip for each dilution. ***Note:*** *The last tube is regarded as a Blank and do not pipette solution into it from the former tube.*



Note: *Each sample addition should preferably be controlled within 10 minutes. To ensure experimental accuracy, it is recommended to test samples in duplicate wells. When pipetting reagents, maintain a consistent order of addition across wells to ensure equal incubation times for all samples.*

C. Preparation of 1x Biotinylated Antibody and 1x Streptavidin-HRP

1. Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use.
2. Dilute solutions to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.

9. Assay Procedure

1. **Reagent Preparation:** Before adding to the wells, equilibrate the TMB substrate for at least 30 mins at room temperature. When diluting samples and reagents, ensure they are mixed completely and evenly. It is recommended to plot a standard curve for each test.

2. **Plate Setup:** Set standard, test sample and control/blank (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
3. **Standard, Samples & Control Loading:** Aliquot 100 µl of standard working solution or samples and controls into the designated wells. ***Note:*** *Add all solutions carefully to the bottom of each micro-ELISA plate well. Avoid touching the inner walls of the wells and minimize foaming during pipetting.*
4. **First Incubation**: Seal the plate with a cover and incubate at 37 °C for 80 mins.
5. **Washing**: Remove the cover, aspirate the liquid from the plate. Wash plate with 200 µl Wash Solution and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Wash plate 3 times. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against absorbent paper.
6. **Biotinylated Antibody Working Solution Addition**: Add 100 µl of Biotin-labelled Antibody working solution to the bottom of each well (standard, test sample & zero wells) without touching the side walls.
7. **Second Incubation**: Seal the plate with a cover and incubate at 37°C for 50 mins.
8. **Washing**: Remove seal and aspirate. Repeat wash process for total 5 times as conducted in step 5.
9. **Addition Streptavidin-HRP Working Solution**: Add 100 µl of Streptavidin-HRP Working Solution into each well, cover the plate and incubate at 37°C for 50 mins.
10. **Washing:** Remove seal and aspirate. Repeat wash process for total 5 times as conducted in step 5.
11. **TMB Substrate Addition and Colour Development**: Add 90 µl of TMB substrate into each well, cover the plate and incubate at 37°C in dark for 20 mins. (***Note****: Do NOT exceed 30 minutes*.) As soon as a blue colour develops preheat the Microplate Reader.
12. **Stop Reaction**: Add 50 µl of Stop solution into each well and mix thoroughly. The colour changes into yellow immediately. ***Note:*** *If colour change does not appear uniform, gently tap the plate to ensure thorough mixing.*

13. **OD Measurement**: Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

10. Data Analysis

Average the duplicate readings for each standard, control, and sample, then subtract the mean optical density of the zero standard. Construct a standard curve by plotting Mouse NGB concentration on the y-axis against absorbance on the x-axis, and fit the data using an appropriate best-fit curve.

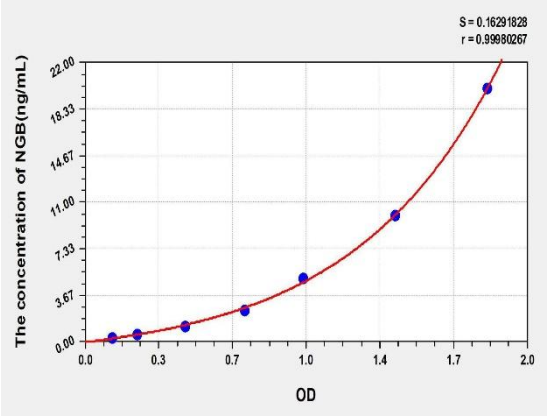
For diluted samples, multiply the concentration obtained from the standard curve by the corresponding dilution factor to determine the final concentration. Data analysis and curve fitting may be performed using suitable plotting software (e.g., CurveExpert).

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

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 Mouse NGB. No significant cross-reactivity or interference between Mouse NGB and other targets was observed.

Note: *Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Mouse NGB and other analytes, therefore, cross reaction may still exist.*

Recovery

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

Matrix	Recovery Range (%)	Average (%)
Serum (n = 5)	92-106%	99%
EDTA Plasma (n = 5)	87-95%	91%
Heparin Plasma (n = 5)	78-92%	85%

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Mouse NGB and their serial dilutions.

Sample	1:2	1:4	1:8	1:16
Serum (n = 5)	85-92%	79-96%	89-101%	85-97%
EDTA Plasma (n = 5)	86-94%	93-102%	89-97%	85-94%
Heparin Plasma (n = 5)	89-99%	83-99%	98-105%	96-101%

Precision

- Intra-Assay: **CV<8%**

  Three samples of known concentration were tested twenty times on 1 plate to assess intra-assay precision.

- Inter-Assay: **CV<10%**

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

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.

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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.