TECHNICAL MANUAL

Human C4 (Complement Component 4) CLIA Kit

- **SKU CODE:** HUES00144
- **SIZE:** 24T/48T/96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 02/2026

Human C4 (Complement Component 4) CLIA 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 And Other Biological Fluids

Reactivity:

Human

Range:

93.75-6000 pg/mL

Sensitivity:

56.25 pg/mL

2. Storage & Expiry

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

3. Product Description

The Assay Genie Human C4 (Complement Component 4) CLIA Kit is a highly sensitive assay for the quantitative measurement of Human C4 in the following samples: Serum, Plasma And Other Biological Fluids.

This kit utilizes a sandwich chemiluminescence Immunoassay (CLIA) 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 CLIA microplate wells, allowing the target to bind to the immobilized antibody. After incubation, a biotinylated detection antibody is then added, which binds specifically to the captured target. Following a wash step to remove excess detection antibody, HRP-conjugated Streptavidin is introduced, forming a biotin-streptavidin-HRP complex. After a second washing step, Substrate Reagent is added to initiate a colorimetric reaction catalyzed by HRP. The reaction produces a blue product that turns yellow upon addition of the acidic Stop Solution.

The Relative Light Units (RLU) is measured by the Chemiluminescence Immunoassay analyzer at 425nm. The RLU 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

No	Component Name	Specifications	Storage
1	Micro CLIA Plate (Dismountable)	96T: 8 wells ×12 strips | 48T: 8 wells ×6 strips | 24T: 8 wells ×3 strips | 96T*5: 5 plates, 96T	-20℃, 6 months
2	Reference Standard	96T: 2 vials | 48T/24T: 1 vial | 96T*5: 10 vials	-20℃, 6 months
3	Concentrated Biotinylated Detection Ab(100×)	96T: 1 vial, 120 µL | 48T/24T: 1 vial, 60 µL | 96T*5: 5 vials, 120 µL	-20℃, 6 months
4	Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL | 48T/24T: 1 vial, 60 µL | 96T*5: 5 vials, 120 µL	-20°C (Protect from light), 6 months
5	Reference Standard & Sample Diluent	96T/48T/24T: 4 vials, 20 mL | 96T*5: 20 vials, 20 mL	2–8°C, 6 months
6	Biotinylated Detection Ab Diluent	96T/48T/24T: 1 vial, 14 mL | 96T*5: 5 vials, 14 mL	2–8°C, 6 months
7	HRP Conjugate Diluent	96T/48T/24T: 1 vial, 14 mL | 96T*5: 5 vials, 14 mL	2–8°C, 6 months
8	Concentrated Wash Buffer(25×)	96T/48T/24T: 1 vial, 30 mL | 96T*5: 5 vials, 30 mL	2–8°C, 6 months
9	Substrate Reagent A	96T/48T/24T: 1 vial, 5 mL | 96T*5: 5 vials, 5 mL	2–8°C (Protect from light)
10	Substrate Reagent B	96T/48T/24T: 1 vial, 5 mL | 96T*5: 5 vials, 5 mL	2–8°C (Protect from light)
11	Plate Sealer	96T/48T/24T: 5 pieces | 96T*5: 25 pieces	-
12	Technical Manual	1 copy	-
13	Certificate of Analysis	1 copy	-
14	Bradford Reagent	96T: 1 vial | 48T: 1 vial	4°C

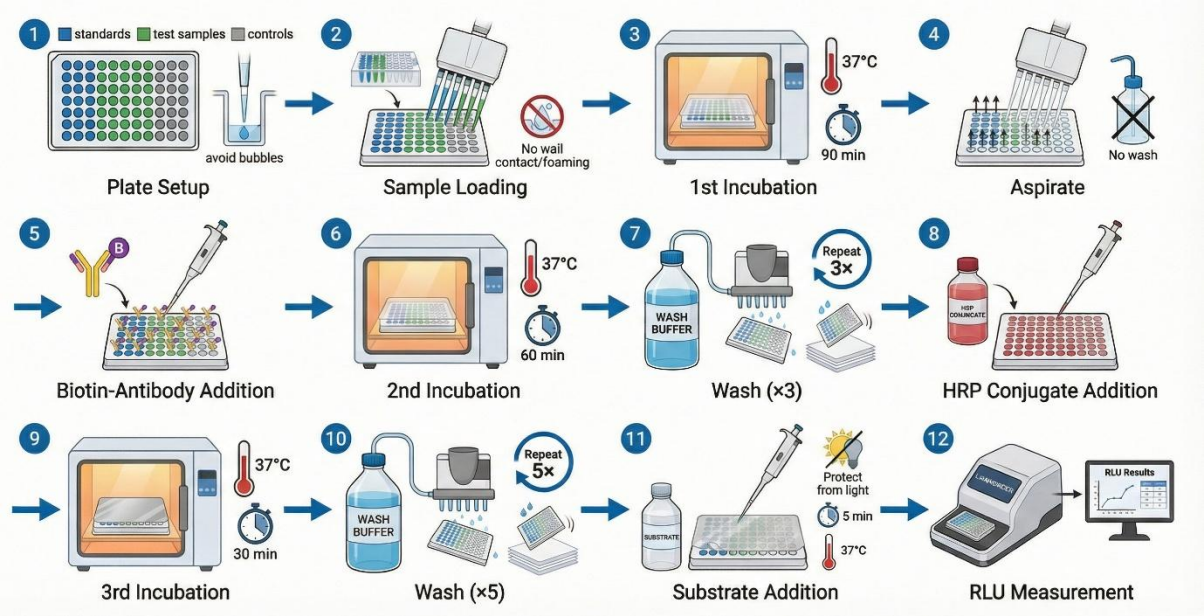
Additional materials required:

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.

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 CLIA 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 CLIA 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 https://www.assaygenie.com/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 350 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 350 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

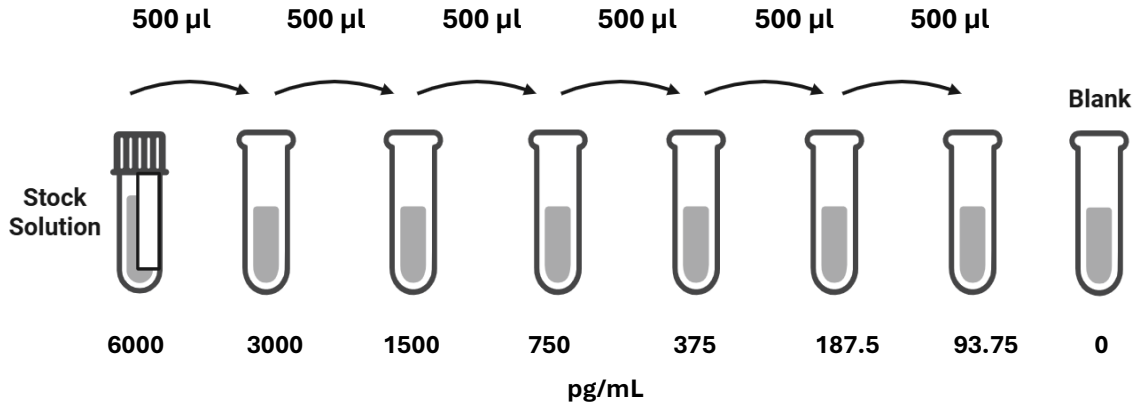
Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

A. Wash Buffer:

Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer (recommended resistivity of ultrapure water is 18MΩ).
Note: *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 the standard tube for 1 min at 10,000 x g.
2. Add 1 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 6000 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 6000, 3000, 1500 750, 375, 187.5, 93.75 and 0 pg/mL. ***Note:*** *The final tube serves as the blank and should not receive any solution transferred from the preceding tube.*
3. Take 7 Eppendorf tubes add 500 µL of Reference Standard & Sample Diluent to each tube. Pipette 500 µL of the 6000 pg/mL working solution to the first tube and mix up to produce a 3000 pg/mL working solution. Pipette 500 µL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.



Note: *The reconstituted standard solution should be aliquoted and stored at −20 °C. It must be used within 2 weeks, and repeated freeze–thaw cycles should be avoided. Gradient-diluted working standards should be freshly prepared immediately before use.*

C. Preparation of Biotinylated Detection Ab working solution:

The working solution should be prepared before starting the experiment.

1. Calculate the required amount before the experiment (100 µL/well). ***Note:*** *It is advisable to prepare an amount marginally exceeding the calculated requirement*.
2. Centrifuge the Concentrated Biotinylated Detection Ab at 800 x g for 1 min.
3. Dilute the 100x Concentrated Biotinylated Detection Ab to 1x working solution with Biotinylated Detection Ab Diluent. (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99)

D. Preparation of HRP Conjugate Working Solution:

The working solution should be prepared before starting the experiment.

1. Calculate the required amount before the experiment (100 µL/well). ***Note:*** *It is advisable to prepare an amount marginally exceeding the calculated requirement*.
2. Centrifuge the Concentrated HRP Conjugate solution at 800 x g for 1 min.
3. Dilute the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

E. Substrate Mixture Solution:

1. Calculate the required amount before the experiment ([PREP_SUBS] µL/well). **Note:** It is advisable to prepare an amount marginally exceeding the calculated requirement.
2. Mix Substrate Reagents A and B in equal volumes immediately before use. Do not open the vials until required. The working solution should be prepared fresh just prior to the assay.

9. Assay Procedure

1. **Plate Setup:** Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. ***Note:*** *Dispense all solutions directly to the bottom of the plate wells, avoiding contact with the well walls. Take care to prevent foaming during the addition of solutions.*
2. **Standard, Samples & Control Loading:** Aliquot 100 µl of µl of standard working solution or samples and controls into the designated wells. **Note:** *Solutions should be added to the bottom of the micro CLIA plate well, avoid touching the inside wall and causing foaming as much as possible.*
3. **First Incubation**: Seal the plate with a cover and incubate at 37 °C for 90 mins.
4. **Aspirate:** Aspirate the liquid from each well, DO NOT wash.
5. **Biotin-labelled Antibody 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.
6. **Second Incubation**: Seal the plate with a cover and incubate at 37°C for 60 mins.
7. **Washing:** Aspirate or decant the solution from the plate and add 350 µL of wash buffer to each well and incubate for 1-2 minutes at room temperature. Aspirate the solution from each well and clap the plate on absorbent filter paper to dry. Repeat this process 3 times. ***Note:*** *A microplate washer can be used in this step and other wash step.*
8. **HRP Conjugate Working Solution Addition:** Add 100 µL of HRP Conjugate working solution to each well.
9. **Third incubation:** Cover with a plate seal and incubate for 30 min at 37°C.
10. **Wash**: Aspirate or decant the solution from each well. Repeat the wash process five times as conducted in step 7.
11. **Substrate Mixture Solution Addition**: Add 100 µl of Substrate Mixture into each well, cover with a new plate sealer. Incubate for no more than 5 min at 37°C. Protect the plate from light.

12. **RLU Measurement**: Determine the RLU value of each well.

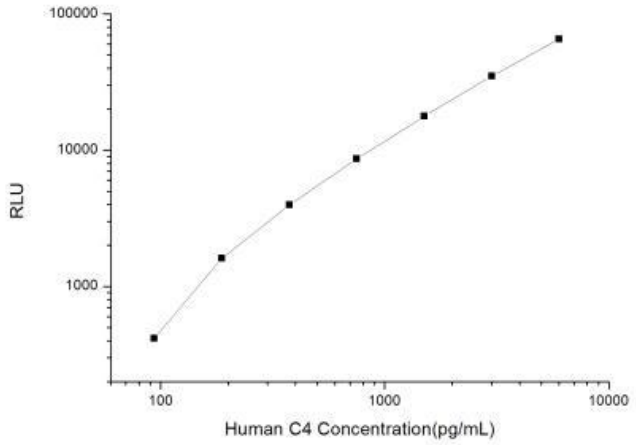
10. Data Analysis

Average the duplicate readings for each standard and sample, then subtract the mean zero-standard optical density. Plot a four-parameter logistic (4-PL) curve on a log–log scale, with standard concentration on the x-axis and RLU values on the y-axis. If a sample's RLU exceeds the upper limit of the standard curve, retest the sample using an appropriate dilution. The final concentration is obtained by multiplying the calculated value by the dilution factor.

11. Typical Data

Standard Curve

Results of a typical standard run of an CLIA 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.



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and high levels were tested 20 times on one plate, (n= replicate).

Inter-assay Precision (Precision between assays): 3 samples with low, mid-range and high level were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	**20**	**20**	**20**	**20**	**20**	**20**
Mean pg/mL	329.42	570.42	2771.77	338.58	604.86	2670.8
Standard deviation	31.29	67.82	167.97	27.26	71.25	198.17
CV (%)	9.5	11.89	6.06	8.05	11.78	7.42

Recovery

Matrices listed below were spiked with a certain level of Human C4 and the recovery rates were calculated by comparing the measured value to the expected amount of Human C4 in the samples.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=8)	93-108	100
EDTA Plasma (n=8)	101-113	108
Cell Culture Media (n=8)	100-115	107

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Human C4 and their serial dilutions.

		Serum (n=5)	EDTA Plasma (n=5)	Cell Culture Media (n=5)
1:2	**Range (%)**	102-116	93-107	88-102
	Average (%)	108	100	94
1:4	**Range (%)**	95-111	97-112	102-114
	Average (%)	102	105	108
1:8	**Range (%)**	89-105	101-118	87-100
	Average (%)	97	110	93
1:16	**Range (%)**	96-108	100-114	97-110
	Average (%)	102	107	103

12. CLIA Troubleshooting

Problem	Possible Causes	Solutions
Poor standard curve	Inaccurate pipetting.	Check pipettes.
	Improper preparation of standard dilutions.	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Incomplete aspiration of wells.	Completely aspirate wells in between steps.
Low Signal	Insufficient incubation time.	Ensure sufficient incubation time.
	Incorrect incubation temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes.	Check pipettes and ensure correct preparation.
	Improper dilutions.	
	HRP conjugate inactive or TMB failure.	Mix HRP conjugate and TMB, rapid coloring.
High color intensity with low RLU/OD	Plate reader setting is not optimal.	Verify the wavelength and filter setting on the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
Large CV	Inaccurate pipetting.	Check pipettes
High background	Concentration of target protein is too high.	Use recommended dilution factor.
	Plate is insufficiently washed.	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer.	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the CLIA kit.	All the reagents should be stored according to the instructions.
	Stop solution is not added.	Stop solution should be added to each well before measurement.

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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.