Technical Manual

Mouse CD4 ColorStep ELISA Kit (120 min)

- Catalogue Code: AEFI03450
- Sandwich ELISA Kit
- Research Use Only

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1. Key features and Sample Types

Aliases:

T-cell surface glycoprotein CD4 ELISA Kit, T-cell surface antigen T4/Leu-3 ELISA Kit, CD4 ELISA Kit

Uniprot:

P06332

Detection method:

Sandwich

Sample Type:

Serum, Plasma, and other biological fluids

Reactivity:

Mouse

Range:

0.391-25 ng/ml

Sensitivity:

0.234 ng/ml

Storage:

See Kit Contents on section 4

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit according to the directions in the kit table of contents on page 5.

3. Description and Principle

The Assay Genie Sandwich ELISA kit is a highly sensitive assay for the quantitative measurement of a specific analyte in the following samples: serum, blood, plasma, cell culture supernatant and other related supernatants and tissues.

How do our ColorStep ELISA kits work?

The Assay Genie ColorStep assay kits are designed for the quantitative measurement of analytes in a wide variety of samples. As today's scientists demand premium quality, consistent data, Assay Genie have developed a range of sensitive, fast, and reliable ELISA kit assays to meet and exceed those demands.

This ColorStep Kit is a quantitative pre-coated ELISA kit with color-coded reagents and is completed in 120 minutes with a 2-wash protocol. A specific anti-tag antibody is pre-coated on the ColorStep plate to capture an affinity-tagged monoclonal antibody that binds to the target analyte. After adding a color-coded antibody mix and samples or standards, a capture–analyte–biotinylated detection antibody complex is formed if Mouse CD4 is present. Following incubation, unbound components are washed away, and HRP-conjugated Streptavidin is added to bind the biotin-labeled detection antibody. After a final wash, TMB substrate is added, producing a blue color that turns yellow upon addition of stop solution. The optical density at 450 nm is measured, and Mouse CD4 concentration is determined using a standard curve. Signal intensity is directly proportional to analyte concentration.

4. Kit Contents

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

No.	Component	Size (48T)	Size (96T)	Storage
1	ELISA Microplate (Dismountable)	8×6	8×12	Place the test strips into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C; Store for 12 months at -20°C.
2	Lyophilized Standard	1 vial	2 vial	Place the standards into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C; Store for 12 months at -20°C.
3	Cap/Det Ab (Ready to use)	3 ml	6 ml	2-8°C (Avoid direct light)
4	HRP-Streptavidin (Ready to use, orange)	5 ml	10 ml	2-8°C (Avoid direct light)
5	TMB Substrate	5 ml	10 ml	2-8°C (Avoid direct light)
6	Sample Dilution Buffer (blue)	20 ml	20 ml	2-8°C
7	Stop Solution	5 ml	5 ml	2-8°C
8	Wash Buffer(25X)	15 ml	30 ml	2-8°C
9	Plate Sealer	3 pieces	5 pieces	-
10	Technical Manual	1 copy	1 copy	-

Please note that the liquid bottles contain slightly more reagent than indicated on the label. Please use a pipette to accurately measure out required amounts.

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.

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plates are kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standards & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross-contamination.

9. Avoid using the reagents from different batches together.

10. Please 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.

Quick Protocol

Step 1: Take out the required plate wells, add 50ul Cap/Det Ab into each well, then add 50ul Standard or Sample into individual well. Gently tap the plate for 10s to ensure thorough mixing then static incubate for 60 minutes at 37°C.

Washing: Wash the plate twice without immersion.

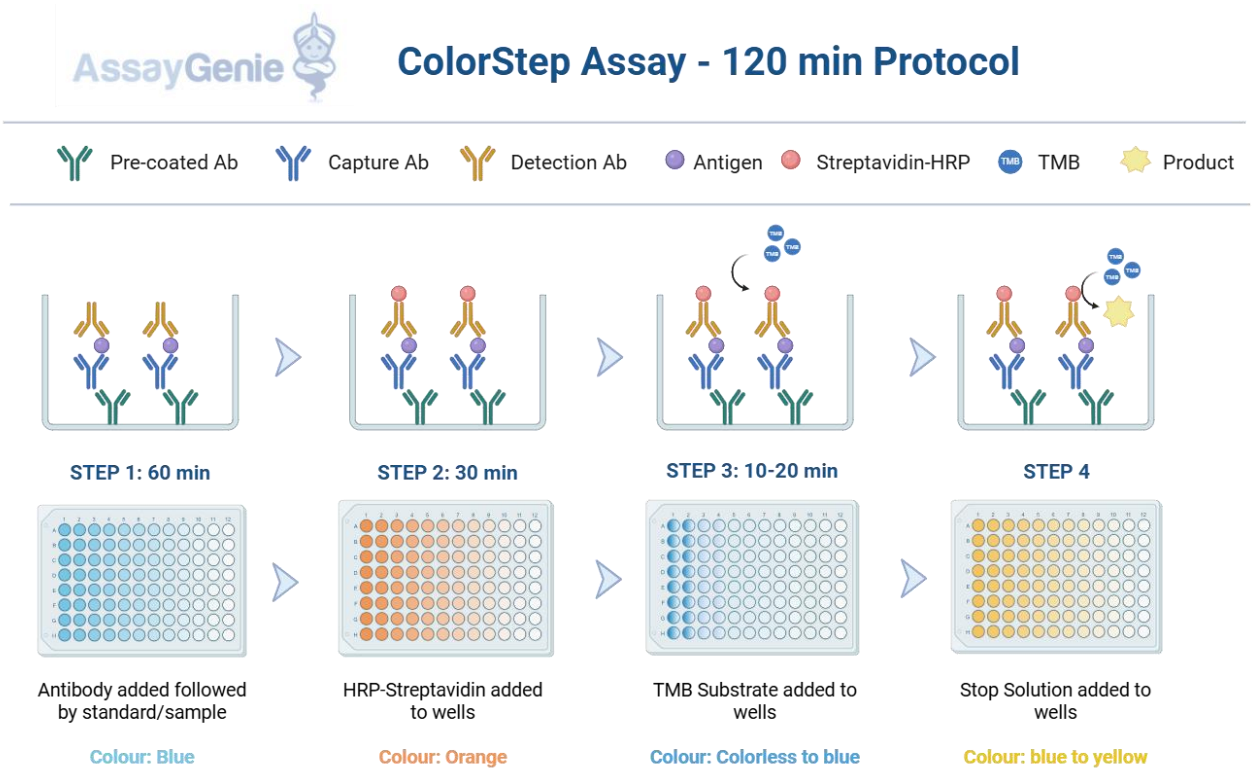
Step 2: Add 100ul HRP-Streptavidin (orange) into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times without immersion.

Step 3: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 4: Add 50ul stop solution. Read at 450nm immediately and calculate**.**

5. Workflow Overview



6. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: Place your whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1,000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future assays.

Plasma: EDTA-Na2/K2 is recommended as the anti-coagulant for this kit. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of sample collection. Once you collect the supernatant you can detect immediately. Alternatively, you can aliquot the supernatant and store it at -20°C or -80°C for future assays. For other anti-coagulant types, please refer to the sample preparation guideline.

Note: Over haemolyzed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1,000 x g. Collect the clear supernatant and assay immediately. Or you can aliquot the supernatant and store it at -80°C for future assays.

Cell lysates:

Suspension Cell Lysate: Centrifuge at your sample at 2,500 rpm for 5 minutes (2-8°C). Add pre-cooled sterile PBS and gently mix. Re-centrifuge your sample as above to collect your cell pellet. Discard PBS following centrifugation, while not disrupting your pellet. Add 0.5-1 ml cell lysis buffer *(NP-40 lysis buffer or Triton X-100 surfactant are not recommended due to their interference with the antibody-antibody reaction)*. Add a suitable protease inhibitor (e.g. PMSF, working concentration: 1 mmol/L). Lyse cells on ice for 30 min - 1 hour. During the lysis process, use a pipette tip or intermittent shaking of the centrifuge tube to completely lyse the cells. Alternatively, subject cells to fragmentation by sonication (14 µM for 30 s) or ultrasonic cell disruptor (300 W, 3~5 s/time. 30 s intervals, four-five times). At the end of the lysis or ultrasonic disruption steps, centrifuge at your sample at 10,000 rpm for 10 minutes (2-8°C). Add the supernatant into a fresh Eppendorf tube and assay immediately, alternatively store at -80°C.

Adherent Cell Lysate: Aspirate your supernatant, followed by washing your cells with pre-cooled sterile PBS three times. Add 0.5-1ml cell lysis buffer and the appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cells with a cell scraper and transfer the sample to a centrifuge tube. Allow the cells to lyse while incubating on ice for 30 min to 1 hour. Alternatively disrupt the cells via sonication as detailed above.

Following cell lysis, centrifuge your samples at 10,000 rpm at 2-8°C for 10 minutes. Then, the supernatant is added into Eppendorf tube and detect immediately. Or you can aliquot the supernatant and store it at - 80°C for future assays.

Tissue Homogenates: Rinse the tissue with 1X Sterile PBS to remove excess blood. Mince tissue after weighing it and homogenize in sterile PBS (the volume depends on the weight of the tissue. 9mL PBS (including protease inhibitors) would be appropriate for 1 gram of tissue). To further lyse your cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5,000 x g and the supernatant is removed for assaying.

Optimal protein concentration for ELISA assays should be within 1-3mg/ml. Some tissue samples such as liver, kidney and pancreas contain a higher endogenous peroxidase

concentration and may react with TMB substrate causing false positivity. In that case, try to use 1% H_2O_2 for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis (pH 7.3) can be used as lysis. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their effects on the kit's performance. We recommend using 50mM Tris, 0.9%NaCL, 0.1%SDS at pH7.3.

Other Biological Samples:

Centrifuge samples for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes for Samples:

- Blood collection tubes should be disposable and endotoxin free. Avoid using haemolyzed and lipemic samples.

- Samples must be stored for less than 5 days at 2-8°C; for 6 months at -20°C; and 2 years at -80°C. Store samples in liquid nitrogen for a longer storage. When melting frozen samples, a quick incubation in a water bath at 15 -25°C can reduce the effect of ice crystals on the samples. After melting, centrifuge to remove any precipitate, and then mix well.

- The detection range of this kit is not equivalent to the concentration of analytes in samples. For analyses with higher or lower concentrations, please properly dilute or concentrate your samples.

- A pre-test is recommended for special samples without reference data to validate their validity.

- Recombinant protein may not match with the capture or detection antibody in this kit and may result in a failed assay.

7. 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).

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: When the concentration of the target in sample is very low, the sample after pre-processed can be added directly 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.

1. Wash Buffer:

Dilute 30 ml (15 ml for 48T) of Concentrated Wash Buffer into 750 ml (375 ml for 48T) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. 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.

2. Standard Dilution:

1) Centrifuge the standard tube for 1 min at 10,000 x g.

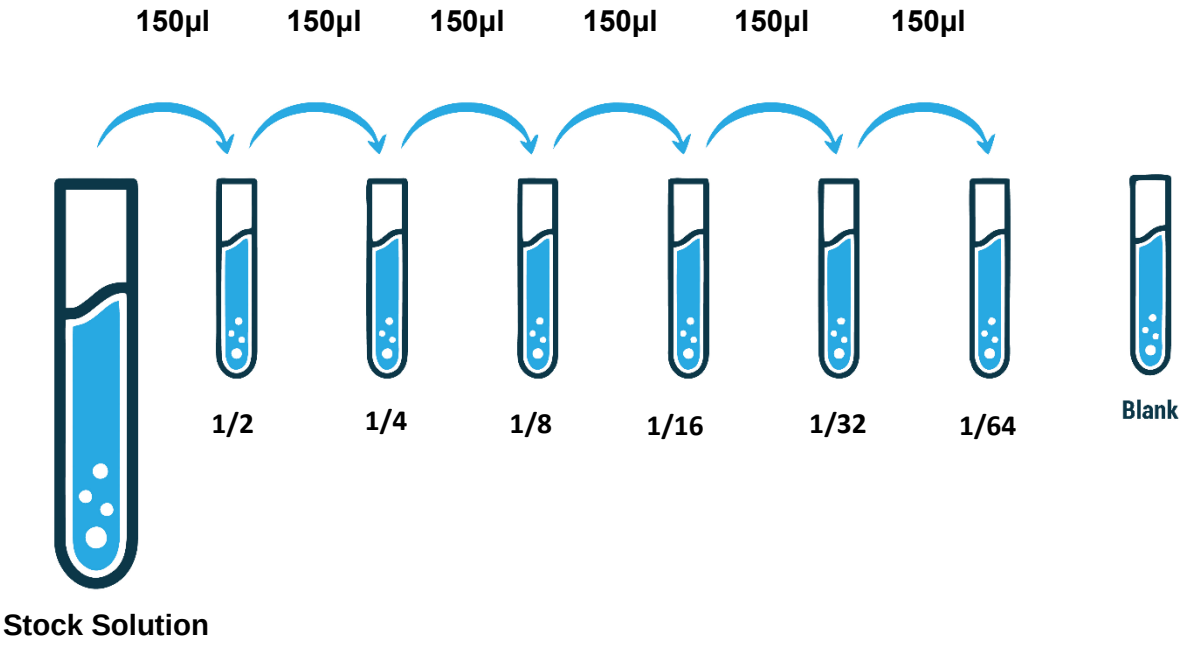
2) Add 0.5ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 10 min. Invert the tube several times to mix (or use a low-speed vortex mixer for 3 – 5 seconds).

3) Finally, centrifuge for 1 min at 1,000 x g to collect liquid at the bottom of the tube and remove bubbles.

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.

4) Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 150µl of the Sample dilution buffer into each tube. Add 150µl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 150µl from 1st tube to 2nd tube and mix thoroughly. Transfer 150µl from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample dilution buffer is used as blank control.

DILUTION SERIES



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.

8. Assay Procedure

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.

1. **Set standard**, **pilot samples, control (blank)** wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.

2. **Cap/Det Ab and Standards/sample loading:** Add 50ul Cap/Det Ab into each well. Aliquot 50ul of zero tube, 1/2 tube, 1/4 tube, 1/8 tube, 1/16 tube, 1/32 tube, 1/64 tube and blank into each standard well. Then, add 50ul pilot samples into sample wells. Immediately, gently tap the plate for 10s to ensure thorough mixing then static incubate for 60 minutes at 37°C. Change the disposable tips for different samples and standards.)

3. **Wash twice:** Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 300-350ul wash buffer into each well without immersion. Discard the liquid from the wells and tap on the absorbent paper again. Repeat the washing step twice.

4. **HRP-Streptavidin**: Add 100ul HRP-Streptavidin into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into room temperature for 30min.)

5. **Wash five times:** Remove the cover and then wash the plate with wash buffer five times. Read washing method in step 3.

6. **TMB Substrate**: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min. (Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.

7. **Stop:** Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.

8. **OD Measurement**: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to

570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. This way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

9. Data Analysis

Calculate the mean OD450 value of the duplicate readings for each standard, control, and sample. Then, obtain the corrected OD450 by subtracting the OD450 blank.

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. (Remove the OD450 blank during plotting.) Alternatively, you can use curve fitting software.

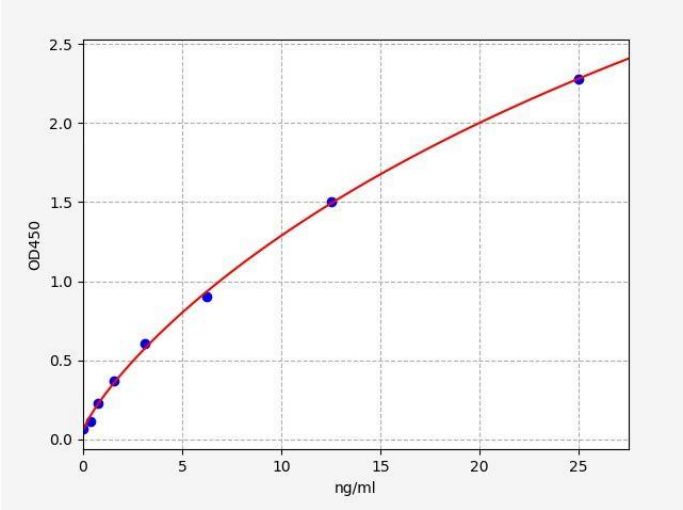
Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

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

10. 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 Mouse CD4. No significant cross-reactivity or interference between Mouse CD4 and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Mouse CD4 and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Mouse CD4 and the recovery rates were calculated by comparing the measured value to the expected amount of Mouse CD4 in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n = 5)	88-99	93
EDTA Plasma (n = 5)	85-105	90
Heparin Plasma (n = 5)	87-98	91

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Mouse CD4 and their serial dilutions.

Sample	1:2	1:4	1:8
Serum (n = 5)	86-101%	84-94%	80-99%
EDTA Plasma (n = 5)	89-100%	83-100%	82-94%
Heparin Plasma (n = 5)	86-103%	83-101%	82-100%

Precision

- **Intra-Assay:** CV<8%
- **Inter-Assay:** CV<10%

Stability

The stability of the Mouse CD4 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**.**

11. ELISA Troubleshooting

If the ELISA result is unsatisfactory, please take a screenshot for the staining result and store the OD data. Keep used strips as well the rest reagents. Contact us to solve your problem with the kit's catalogue number and batch number. You can also refer to the following table to check the reason.

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect order for adding reagents	Confirm the required reagent added in each step. Also repeat the assay and verify.
	Components used from different kits	Use the component included in the same kit. Also repeat the assay and verify.
	Forget to add some reagents	Verify whether the required reagent is added.
Overflow OD	Use components from different kits, or prepare the working solution with higher concentration	Use the component included in the same kit. Also repeat the assay and verify.
Poor standard curve	Inappropriate curve fitting model	Try to plot the curve by different fitting models.
	The amount of sample is lower than the detection range.	Decrease dilution ratio or concentrate the sample.

Problem	Possible Causes	Solutions
Samples without signal	The detection target is incompatible with the buffer.	Verify the compatibility of sample storage buffer with the sample.
	Incorrect preparation of sample	Please refer to sample preparation guideline.
	Longer storage of sample or freeze- thaw cycle	Aliquot and store samples according to the assay requirement.
High CV%	Precipitate is formed in the well during staining.	Increase the dilution ratio of the sample.
	Unclean plate	Don't touch the bottom of the plate during the assay.
	Foam is found in the well.	Avoid foaming during reading in a microplate reader.
	Each well is washed unevenly.	Check whether the tube of the washer is smooth.
	Reagents are not completely mixed.	Mix all reagents completely.
	Inconsistent pipetting	Use calibrated pipette and correct pipetting method.
Standard curve with low signal	Standards are improperly reconstituted.	Before opening, shortly centrifuge the lyophilized standard tube till complete dissolution.
	Standards have been degraded.	Follow suggested storage conditions for

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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.

Contact Details

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