resDetect™ Human VEGF165 ELISA Kit (Residue Testing)

Pack Size: 96 tests

Catalog Number: RES-A121

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

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【Product Overview】

resDetect™ Human VEGF165 ELISA Kit (Residue Testing) is used to detect and quantitatively determine Human VEGF165 residues in cell culture supernatants. The kit is compatible with GMP Human VEGF165 Protein (ACROBiosystems, cat. GMP-VE5H23) to ensure accurate assay results and is designed to provide a reliable solution for cell therapy product quality assessment during drug development and CMC quality control stages. It can also be used as a universal detection tool for the quantitative determination of Human VEGF165.

【Assay Principle】

This kit quantifies VEGF165 using a sandwich ELISA format. The microplate is pre-coated with an Anti-VEGF165 Antibody, which captures VEGF165 present in standards and samples. After washing, a Biotin-Anti-VEGF165 Antibody is added to bind the captured VEGF165, forming an Antibody-antigen-biotinylated antibody sandwich complex. After washing, a Streptavidin-HRP is added to the plate. Following additional washes, a substrate is added for color development. The reaction is stopped with stop solution, and the color changes from blue to yellow. Absorbance is measured at 450 nm with a 630 nm reference. The absorbance signal is directly proportional to the VEGF165 concentration in the sample.

【Precautions】

1. **For research use only**. Not for use in diagnostic or therapeutic procedures.
2. **Use before the expiration date** indicated on the label.
3. Do not mix or interchange reagents from different lots or kits.
4. If a sample OD exceeds the highest standard dilute the sample with Dilution Buffer and retest.
5. Result variability may arise from operator technique, equipment performance, incubation conditions, or storage/handling. Follow the protocol exactly. The kit is designed to reduce some endogenous interference in biological samples. However, not all potential interfering factors can be eliminated.

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【Materials Provided】

Table 1. Materials Provided

ID	Components	Size (96 T)	Format	Storage	
				Unopened	Opened
RES121-C01	Pre-coated Anti-VEGF165 Antibody Microplate	1 plate	Solid	2-8℃	2-8℃
RES121-C02	Human VEGF165 Standard	20 μg	Powder	2-8℃	-70℃
RES121-C03	Biotin-Anti-VEGF165 Antibody	20 μg	Powder	2-8℃	-70℃
RES121-C04	Streptavidin-HRP	50 μL	Liquid	2-8℃, Protected from light	2-8℃, Protected from light
RES121-C05	10×Washing Buffer	50 mL	Liquid	2-8℃	2-8℃
RES121-C06	Dilution Buffer	50 mL	Liquid	2-8℃	2-8℃
RES121-C07	Substrate Solution	12 mL	Liquid	2-8℃, Protected from light	2-8℃, Protected from light
RES121-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

Note: Briefly centrifuge the Streptavidin-HRP before use to bring the contents to the bottom of the tube.

【Storage】

1. Store the unopened kit at 2-8°C upon receipt.
2. Locate the expiration date on the outer packaging and do not use reagents beyond their expiration date.

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【Reagents and Consumables / Equipment Required but not Provided】

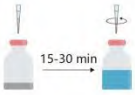
Table 2. Reagents and Consumables / Equipment Required but not Provided

Items	Specification
Deionized or distilled water	/
Single- or multi-channel micropipettes	Calibrated
Low-retention pipette tips	10 µL, 100 µL, 300 µL, 1000 µL
Reagent bottle	500 mL
Centrifuge tubes	1.5 mL, 10 mL
Microplate shaker	For 96-well plate shaking
Vortex mixer	/
Timer	/
Incubator	37°C
Microplate reader	Single- or dual- wavelength microplate reader capable of measuring absorbance at 450 nm with a 630 nm reference in 96-well microplates.

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【Quick Guide】

Step 1 Preparation of the Standard Curve



Standard reconstitution: Add ultrapure water, let stand for 15-30 min at room temperature, then mix thoroughly by pipetting.

Prepare the serial dilutions:

Tubes/Solution Code	Standard Stock Solution	Std.-0	Std.-1'	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6
Procedure	10 µL	20 µL	30 µL	300 µL	300 µL	300 µL	300 µL	300 µL	
Solution Conc.	100 µg/mL	2000 ng/mL	80 ng/mL	4000 pg/mL	2000 pg/mL	1000 pg/mL	500 pg/mL	250 pg/mL	125 pg/mL
Dilution Buffer Vol.		490 µL	480 µL	570 µL	300 µL	300 µL	300 µL	300 µL	300 µL

Step 2 Add standards, samples, and blank controls



Addition: Add 100 µL of standards, samples, and blank to the designated wells.



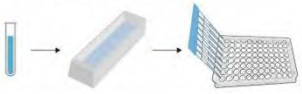
Incubation: Seal the plate and incubate at room temperature for 1.0 h.

Step 3 Add Detection Antibody



Washing (manual or automated): Discard the liquid. Add 300 µL of wash buffer, wash 3 times, and tap dry.

Addition: Add 100 µL to each well.





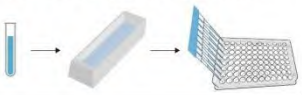
Incubation: Seal the plate and incubate at room temperature for 1.0 h.

Step 4 Add Streptavidin-HRP



Washing (manual or automated): Discard the liquid. Add 300 µL of wash buffer, wash 3 times, and tap dry.

Addition: Add 100 µL to each well.





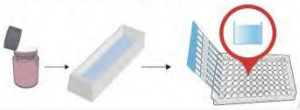
Incubation: Seal the plate and incubate at room temperature for 1.0 h.

Step 5 Add Substrate Solution



Washing (manual or automated): Discard the liquid. Add 300 µL of wash buffer, wash 3 times, and tap dry.

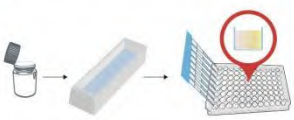
Addition: Add 100 µL to each well.





Incubation: Seal the plate and incubate at room temperature for 20 min, protected from light.

Step 6 Add Stop Solution and Data Recording



Addition: Add 50 µL of Stop Solution.
Note: The color will change from blue to yellow.



Data Recording: Measure the absorbance at 450 nm with a 630 nm reference within 10 min after adding the stop solution.

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【Reagent Preparation】

Bring all reagents to room temperature (20-25°C) before use. If crystals are present in the solution, allow the reagents to equilibrate until the crystals are completely dissolved. If needed, incubate at 37°C for 10-15 minutes to facilitate dissolution.

According to Table 3, reconstitute the provided lyophilized product with ultrapure water to prepare the stock solution. Allow the vial to stand at room temperature for 15 to 30 minutes, then gently pipette up and down to mix. Do not vortex or shake vigorously.

Store the reconstituted stock solution at -70°C. It is recommended to aliquot the stock solution to avoid repeated freeze-thaw cycles. Do not exceed one freeze-thaw cycle. Each aliquot should contain at least 4 μg of material.

Note: Due to unavoidable batch-to-batch variability in protein quantification, if lower back-calculation error is desired, users may use the same production batch of the protein as the standard to generate the standard curve for the corresponding residual analysis.

Table 3. Preparation Method

ID	Components	Format (96 T)	Concentration	Reconstituted water Vol.
RES121-C02	Human VEGF165 Standard	20 μg	100 μg/mL	200 μL
RES121-C03	Biotin-Anti-VEGF165 Antibody	20 μg	100 μg/mL	200 μL

【Assay Procedure】

1. Preparation of Working Solution

1.1 Preparation of 1×Washing Buffer

Dilute 50 mL of 10×Washing Buffer with ultrapure water or deionized water to a final volume of 500 mL and mix gently.

1.2 Preparation of Biotin-Anti-VEGF165 Antibody working solution

Dilute the Biotin-Anti-VEGF165 Antibody to 0.01 μg/mL using Dilution Buffer. Prepare fresh before use.

1.3 Preparation of Streptavidin-HRP working solution

Dilute the Streptavidin-HRP at 1:4000 using Dilution Buffer. Prepare fresh before use, protected from light.

2. Preparation of the Standard Curve

The concentration of the reconstituted Human VEGF165 Standard (RES121-C02) is 100 μg/mL.

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2.1 Prepare Std.-0

Dilute 10 µL of the reconstituted Human VEGF165 Standard into 490 µL of Dilution Buffer. Mix gently and thoroughly.

2.2 Prepare Std.-1'

Dilute 20 µL of Std.-0 into 480 µL of Dilution Buffer. Mix gently and thoroughly.

2.3 Prepare the highest standard (Std.-1, 4000 pg/mL)

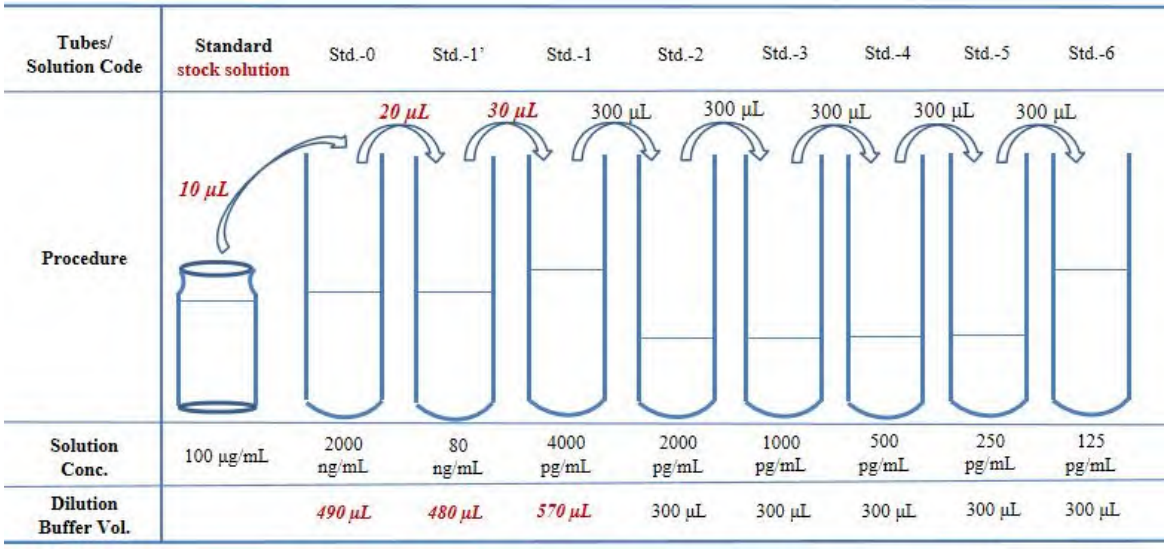
Dilute 30 µL of Std.-1' into 570 µL of Dilution Buffer. Mix gently and thoroughly.

2.4 Prepare the Serial Dilutions

Add 300 µL of Dilution Buffer to each of the remaining centrifuge tubes. Perform 2-fold serial dilutions starting from Std.-1 to generate the standard curve. Mix thoroughly at each dilution step. Dilution Buffer serves as the zero standard (Blank). The dilution procedure is shown in Figure 1.

Note: For residual analysis, it is recommended to generate the standard curve using standards from the same manufacturer and production lot as the target analyte to minimize measurement variability caused by differences in quantification methods or other supplier-related factors.

Figure 1. Preparation of 2-Fold Serial Dilutions of the Human VEGF165 Standard



3. Preparation of Samples (For reference)

Spike recovery experiments are a critical component of ELISA analysis. They are designed to assess potential interference from the sample matrix on target analyte detection and verify the reliability of the assay method. The core principle of this approach is to spike a known quantity of standard material into samples with a pre-determined concentration; recovery is then calculated by comparing the measured signal increment to the theoretical amount of standard added.

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3.1 Pretreatment of Test Samples

Serially dilute the test samples with Dilution Buffer to ensure that the analyte concentration falls within the detection range of the standard curve and to reduce potential matrix interference. The dilution scheme is shown in Table 4.

Table 4. Preparation Method

Sample ID	Volume(μL)	Dilution Buffer(μL)	Total volume(μL)	Final Dilution Ratio	Diluted Sample ID
Test sample	150	150	300	2	Test sample-1
Test sample-1	150	150	300	4	Test sample-2

Note: 1) The required dilution factor may vary depending on the sample matrix and should be optimized for each sample type.

2) Perform serial dilutions carefully to ensure accuracy. It is recommended that the dilution factor for any single step does not exceed 10-fold, as excessive dilution may affect assay accuracy.

3.2 Preparation of Spiking Working Solution

Based on the expected sample concentrations, dilute the standard to an appropriate concentration for the spiking working solution using Dilution Buffer. Prepare the spiking solution freshly before use.

Note: The final concentration of analyte in the spiked samples should fall within the linear detection range of the assay to avoid inaccurate results caused by values outside the assay range.

3.3 Preparation of Spiked Samples

Mix the test samples prepared in Step 3.1 with either the spike working solution or Dilution Buffer at a 1:1 (v/v) ratio to prepare spiked and unspiked samples, respectively. Mix thoroughly. The dilution scheme is shown in Table 5.

Note: To minimize procedural variation, ensure that the sample volume, diluent or spike volume, mixing method, incubation time, and incubation temperature are identical for both spiked and unspiked samples.

Table 5. Preparation Method

Sample ID	Group	Volume(μL)	Dilution Buffer(μL)	Spiking working solution(μL)	Total volume(μL)	Final Dilution Ratio
Test sample	Unspiked	150	150	0	300	2
Test sample	Spiked	150	0	150	300	2
Test sample-1	Unspiked	150	150	0	300	4
Test sample-1	Spiked	150	0	150	300	4

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Test sample-2	Unspiked	150	150	0	300	8
Test sample-2	Spiked	150	0	150	300	8

4. Addition of Samples

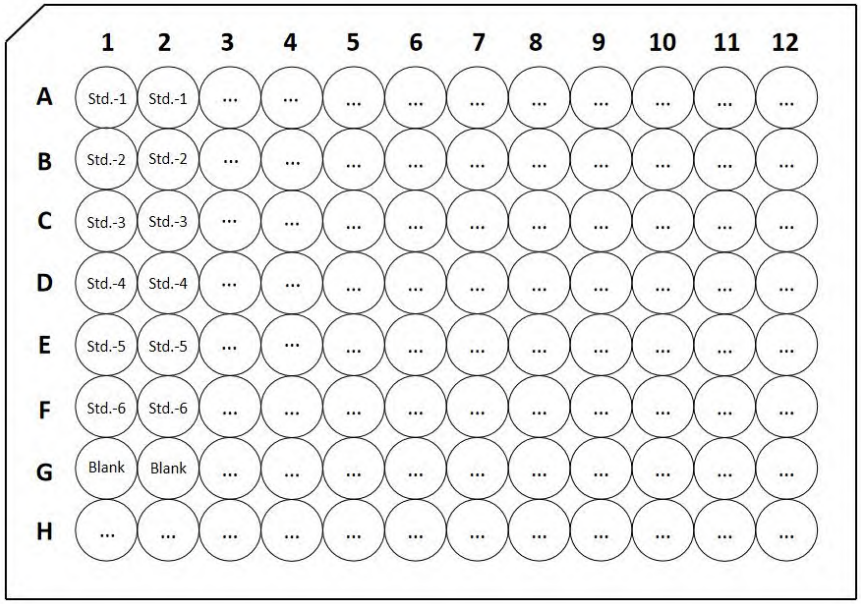
Add 100 µL serially diluted standards, test samples and spiked samples to the appropriate wells. Add 100 µL of Dilution Buffer to the blank control wells.

Note: 1) It is recommended to run all standards and samples in duplicate wells.

2) The blank control contains Dilution Buffer only.

3) It is recommended to use the example plate layout shown in Figure 2 to record the positions of standards and samples.

Figure 2. Recommended Plate Layout for Standards and Samples



5. Incubation

Seal the plate with microplate sealing film. Incubate at room temperature for 1.0 hour.

6. Washing

Carefully remove the plate sealer and discard the liquid from the wells. Add 300 µL of 1×Washing Buffer to each well and soak for 10 seconds. Aspirate or decant the buffer. Repeat for a total of three washes. After the final wash, invert the plate and blot dry on absorbent paper.

7. Addition of Detection Antibody

Add 100 µL Biotin-Anti-VEGF165 Antibody working solution (0.01 µg/mL) to each well. The working solution should be prepared fresh before use.

8. Incubation

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Seal the plate with microplate sealing film. Incubate at room temperature for 1.0 hour.

9. Washing

Repeat step 6.

10. Addition of Streptavidin-HRP

Add 100 µL Streptavidin-HRP working solution (1:4000 dilution) to each well. Prepare the working solution fresh before use.

11. Incubation

Seal the plate with microplate sealing film. Incubate at room temperature for 1.0 hour.

12. Washing

Repeat step 6.

13. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate and incubate at room temperature for 20 minutes, protected from light.

14. Reaction Termination

Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

Note: The color in the wells will change from blue to yellow.

15. Data Recording

Measure the absorbance at 450 nm with a 630 nm reference within 10 minutes after adding the stop solution.

Note: Subtracting the OD_{630nm} value from the OD_{450nm} value helps reduce background interference.

【Calculation of Results】

1. Calculate the mean absorbance of duplicate wells for each standard and sample. Subtract the absorbance of the zero standard (Blank) from each value ($OD_{450\ nm}$-$OD_{630\ nm}$-Blank).
2. Generate the standard curve by plotting the standard concentrations on the x-axis and the corrected absorbance values on the y-axis. Fit the curve using a four-parameter logistic (4-PL) model. The coefficient of determination (R^2) should be ≥ 0.9900.
3. Determine the concentrations of the samples and spiked samples from the standard curve. Multiply the calculated concentrations by the appropriate dilution factors.
4. Calculation of spiked recovery (%)

$$\text{Recovery Rate (\%)} = \frac{\text{Concentration}_{\text{ Spiked Sample}} - \text{Concentration}_{\text{ Unspiked Sample}}}{\text{Concentration}_{\text{ Control Spike}}} \times 100\%$$

If the spike recovery falls outside the range of 80%-120%, this may indicate matrix interference

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affecting the ELISA assay. In such cases, optimize sample pre-treatment (e.g., additional dilution) before retesting.

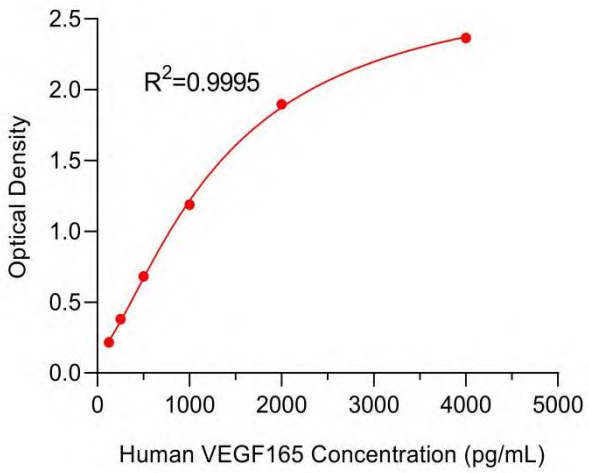
5. Detection Range

The assay detection range is 125 pg/mL-4000 pg/mL. Samples with concentrations above the upper limit of the calibration curve should be reported as >4000 pg/mL or diluted appropriately and reanalyzed to fall within the linear range. Samples with concentration below the lower limit of calibration curve should be reported as < 125 pg/mL.

【Typical Data】

A standard curve must be generated for each microplate in every experiment. Absolute OD values may vary depending on the laboratory, operator, and equipment used. The example data provided below are for reference only.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
4000	2.411	2.411	2.411	2.365
2000	1.956	1.931	1.944	1.898
100	1.233	1.237	1.235	1.189
500	0.736	0.717	0.727	0.681
250	0.410	0.441	0.426	0.380
125	0.265	0.256	0.261	0.215
0	0.073	0.077	0.075	/



【Sensitivity】

The minimum detectable concentration (MDC) of VEGF165 is 35.801 pg/mL. The MDC was determined by calculating the mean optical density (OD) of 20 zero-standard replicates, adding two standard deviations (mean+2SD), and converting the resulting OD value to concentration using the standard curve.

【Precision】

1. Intra-assay Precision

Three samples of known concentration were tested in ten replicates within a single plate to evaluate intra-assay precision.

2. Inter-assay Precision

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Three samples of known concentration were tested in three independent assays to evaluate inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	2826.441	1389.337	318.605	2791.107	1412.683	316.569
SD	174.683	93.731	22.376	32.192	29.958	4.914
CV (%)	6.2	6.7	7.0	1.2	2.1	1.6

【Recovery】

Five individual blank T cell culture supernatant samples were spiked with human VEGF165 at different concentrations. The blank T cell culture supernatant without VEGF165 was used as the blank matrix control for recovery calculation. The recovery ranged from 89.1% to 112.4%, with an average recovery of 98.2%.

Sample(n=5)	Average Recovery (%)	Range (%)
T cell culture supernatant	98.2	89.1-112.4

【Linearity】

To assess the assay linearity, a high-concentration standard was spiked into different dilution matrices and serially diluted to generate concentrations within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (RPMI 1640)
1:2	Average Recovery (%)	95.3	98.7
	Range (%)	87.5-105.6	89.2-115.0
1:4	Average Recovery (%)	99.4	96.8
	Range (%)	89.9-109.8	85.7-102.8
1:8	Average Recovery (%)	114.5	108.2
	Range (%)	104.8-118.9	100.5-117.4
1:16	Average Recovery (%)	112.2	114.5
	Range (%)	110.3-114.2	112.7-116.6

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【Specificity】

This assay detects both natural and recombinant VEGF165. No cross-reactivity was observed when the kit was tested against the following recombinant cytokines.

Human		
IL-2	IL-7	IL-15
FGF	EGF	GM-CSF

【Interfering Substances】

Potential matrix interference was evaluated by adding various concentrations of DMSO and HSA to the sample diluted buffer.

Additive	Tolerated concentration
DMSO	2%
HSA	5%

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【Troubleshooting Guide】

Problem	Cause	Solution
Low signal	a. Kit components were not equilibrated to room temperature before use; b. Insufficient reconstitution time for lyophilized components.	a. Remove the kit from 2-8 °C storage in advance and allow all reagents to fully equilibrate to room temperature before starting the assay; b. After adding reconstitution buffer, allow lyophilized components to stand for at least 15 minutes and mix gently before use.
Poor assay reproducibility	a. Improper storage of reagents after opening; b. In consistent timing during sample dilution of pipetting.	a. Store reagents strictly according to the instructions in this manual; b. Plan the experimental workflow in advance and properly schedule assay timing.
High background	a. Insufficient washing; b. Excessive incubation temperature or prolonged substrate development time; c. Reagent contamination.	a. Increase soak time during wash steps and ensure the plate is thoroughly blotted dry after final wash; b. Strictly follow the operating procedures described in the manual; c. Use clean reagents and consumables, and maintain a clean experimental environment.
Edge effects	Uneven temperature distribution across the plate.	Ensure uniform incubation temperature and avoid stacking plates during incubation.
Good standard curve but no detectable signal in samples	a. Interfering substances present in the samples; b. Target analyte concentration below the assay detection.	a. Optimize sample dilution to minimize matrix interference; b. Use a higher-sensitivity assay if lower analyte levels are expected.

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