resDetect™ Human GDNF ELISA Kit (Residue Testing)

Pack Size: 96 tests

Catalog Number: RES-A117

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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Content

【Product Overview】 1

【Assay Principle】 1

【Precautions】 1

【Materials Provided】 2

【Storage】 2

【Reagents and Consumables / Equipment Required but not Provided】 3

【Quick Guide】 4

【Reagent Preparation】 5

【Assay Procedure】 5

【Calculation of Results】 10

【Typical Data】 10

【Sensitivity】 11

【Precision】 11

【Recovery】 11

【Linearity】 12

【Specificity】 12

【Interfering Substances】 12

【Troubleshooting Guide】 13

【Product Overview】

GDNF (Glial Cell Line-Derived Neurotrophic Factor) is a critical neurotrophic factor vital for neuronal survival, differentiation, and functional maintenance. It plays a key role in central nervous system diseases (such as Parkinson's, spinal cord injury), neural regeneration, and neurodegenerative disorder research. Given its importance in multiple research areas, accurate detection of GDNF residues is essential for exploring its biological functions and therapeutic applications. The kit is compatible with GMP Human GDNF Protein (ACROBiosystems, cat# GMP-GDFH17) and premium grade Human GDNF Protein (ACROBiosystems, cat# GDF-H5118) to ensure assay accuracy, designed to offer a reliable solution for quality assessment of cell therapy products in drug development and CMC quality control. This ELISA kit is developed to efficiently and precisely detect GDNF residues, providing a trustworthy tool for scientific research and potential clinical use.

【Assay Principle】

This kit quantifies GDNF using a sandwich ELISA format. The microplate is pre-coated with an Anti-GDNF Antibody, which captures GDNF present in standards and samples. After washing, a Biotin-Anti-GDNF Antibody is added to bind the captured GDNF, 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 GDNF 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 1×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
RES117-C01	Pre-coated Anti-GDNF Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES117-C02	Human GDNF Standard	20 μg	Powder	2-8°C	-70°C
RES117-C03	Biotin-Anti-GDNF Antibody	20 μg	Powder	2-8°C	-70°C
RES117-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, Protected from light	2-8°C, Protected from light
RES117-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES117-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES117-C07	Substrate Solution	12 mL	Liquid	2-8°C, Protected from light	2-8°C, Protected from light
RES117-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

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	10 µL	10 µL	300 µL	300 µL	300 µL	300 µL	300 µL	
Solution Conc.	200 µg/mL	2000 ng/mL	20 ng/mL	200 pg/mL	100 pg/mL	50 pg/mL	25 pg/mL	12.5 pg/mL	6.25 pg/mL
Dilution Buffer Vol.		990 µL	990 µL	990 µL	300 µL	300 µL	300 µL	300 µL	300 µL

Step 2 Add standards, samples, and blank controls

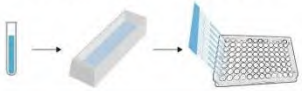
Addition: Add 100 µL of standards, samples, and blank to the designated wells.

Incubation: Seal the plate and incubate at room temperature with shaking at 400 rpm 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.

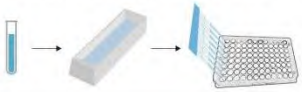


Incubation: Seal the plate and incubate at room temperature with shaking at 400 rpm for 1.0 h.

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.

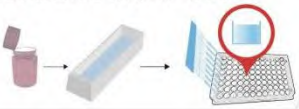


Incubation: Seal the plate and incubate at room temperature with shaking at 400 rpm for 1.0 h.

Step 5 Add Substrate Solution

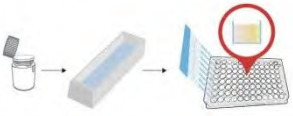
Washing (manual or automated): Discard the liquid. Add 300 µL of wash buffer, wash 3 times, and tap dry.

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.
RES117-C02	Human GDNF Standard	20 μg	200 μg/mL	100 μL
RES117-C03	Biotin-Anti-GDNF Antibody	20 μg	200 μg/mL	100 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 1×Dilution Buffer

Dilute 50 mL of 2×Dilution Buffer with 1×Washing Buffer to a final volume of 100 mL and mix gently.

1.3 Preparation of Biotin-Anti-GDNF Antibody working solution

Dilute the Biotin-Anti-GDNF Antibody to 0.1 μg/mL using 1×Dilution Buffer. Prepare fresh before use.

1.4 Preparation of Streptavidin-HRP working solution

Dilute the Streptavidin-HRP at 1:2500 using 1×Dilution Buffer. Prepare fresh before use, protected from light.

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2. Preparation of the Standard Curve

The concentration of the reconstituted Human GDNF Standard (RES117-C02) is 200 μg/mL.

2.1 Prepare Std.-0

Dilute 10 μL of the reconstituted Human GDNF Standard into 990 μL of 1×Dilution Buffer. Mix gently and thoroughly.

2.2 Prepare Std.-1'

Dilute 10 μL of Std.-0 into 990 μL of 1×Dilution Buffer. Mix gently and thoroughly.

2.3 Prepare the highest standard (Std.-1, 200 pg/mL)

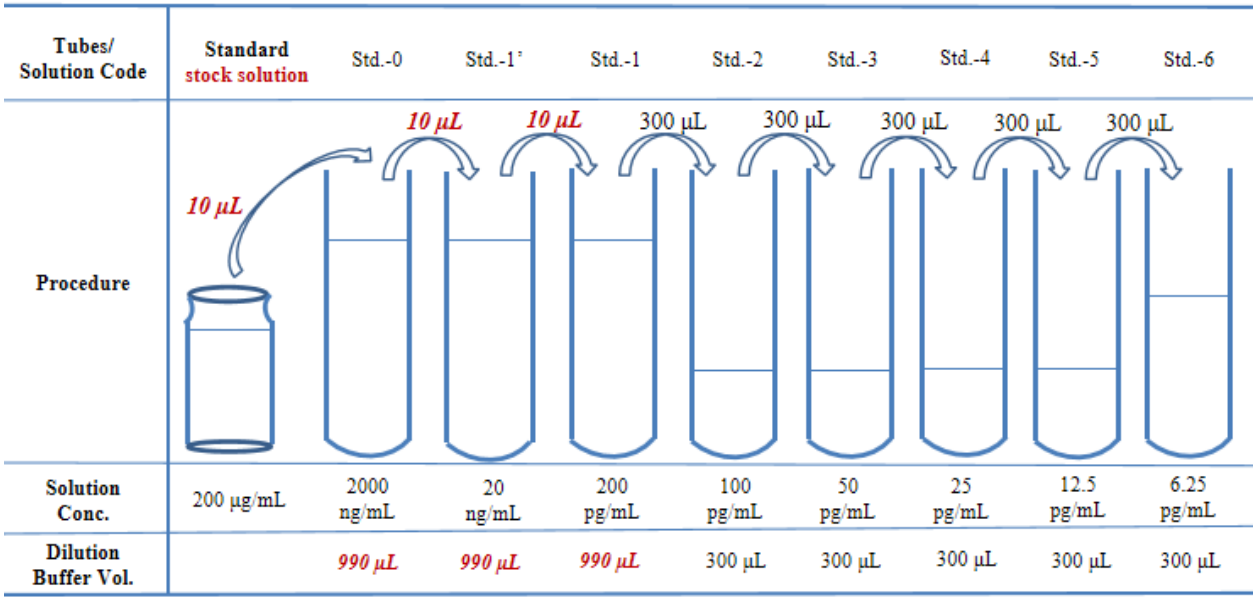
Dilute 10 μL of Std.-1' into 990 μL of 1×Dilution Buffer. Mix gently and thoroughly.

2.4 Prepare the Serial Dilutions

Add 300 μL of 1×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. 1×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 GDNF 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

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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.

3.1 Pretreatment of Test Samples

Serially dilute the test samples with 1×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)	1×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 1×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 1×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.

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Table 5. Preparation Method

Sample ID	Group	Volume(μL)	1×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
Test sample-2	Unspiked	150	150	0	300	8
Test sample-2	Spiked	150	0	150	300	8

4. Addition of Samples

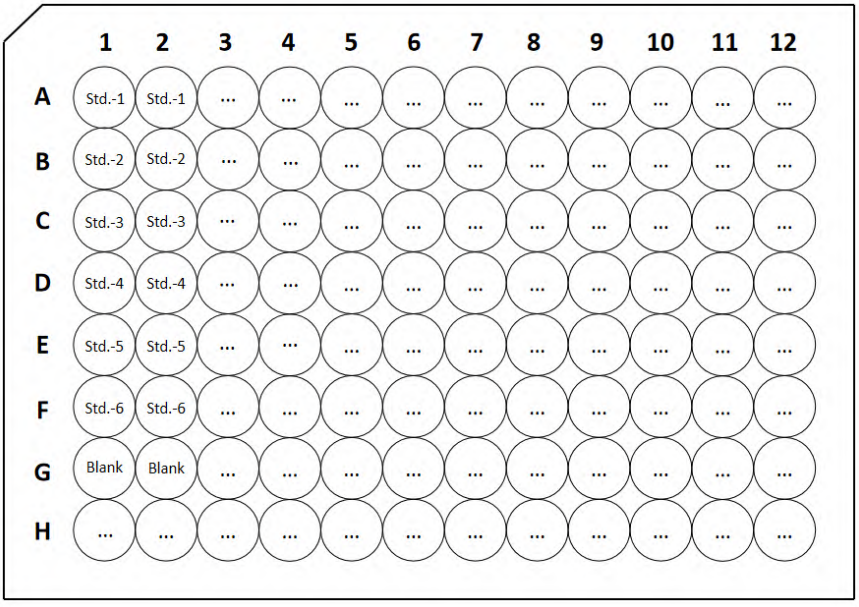
Add 100 μL serially diluted standards, test samples and spiked samples to the appropriate wells. Add 100 μL of 1×Dilution Buffer to the blank control wells.

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

2) The blank control contains 1×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 on a microplate shaker set to 400 rpm for 1.0 hour.

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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-GDNF Antibody working solution (0.1 µg/mL) to each well. The working solution should be prepared fresh before use.

8. Incubation

Seal the plate with microplate sealing film. Incubate at room temperature on a microplate shaker set to 400 rpm for 1.0 hour.

9. Washing

Repeat step 6.

10. Addition of Streptavidin-HRP

Add 100 µL Streptavidin-HRP working solution (1:2500 dilution) to each well. Prepare the working solution fresh before use.

11. Incubation

Seal the plate with microplate sealing film. Incubate at room temperature on a microplate shaker set to 400 rpm 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.

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【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 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 6.25 pg/mL-200 pg/mL. Samples with concentrations above the upper limit of the calibration curve should be reported as >200 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 < 6.25 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.

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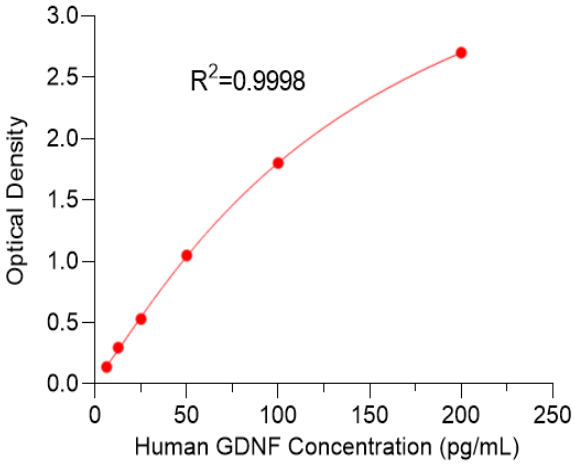
Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
200	2.719	2.736	2.728	2.702
100	1.928	1.730	1.829	1.803
50	1.100	1.049	1.075	1.049
25	0.556	0.556	0.556	0.530
12.5	0.328	0.319	0.324	0.298
6.25	0.175	0.154	0.165	0.139
0	0.028	0.024	0.026	/



【Sensitivity】

The minimum detectable concentration (MDC) of GDNF is 0.479 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 ten replicates within a single plate to evaluate intra-assay precision.

2. Inter-assay Precision

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)	151.662	39.517	15.443	149.825	38.895	15.670
SD	4.800	1.166	0.617	4.598	1.076	0.474
CV (%)	3.2	3.0	4.0	3.1	2.8	3.0

【Recovery】

Five individual blank T cell culture supernatant samples were spiked with human GDNF at different concentrations. The blank T cell culture supernatant without GDNF was used as the blank matrix

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control for recovery calculation. The recovery ranged from 87.6% to 105.3%, with an average recovery of 95.9%.

Sample(n=5)	Average Recovery (%)	Range (%)
T cell culture supernatant	95.9	87.6-105.3

【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 (%)	91.1	101.4
	Range (%)	86.3-97.7	98.5-104.4
1:4	Average Recovery (%)	93.6	101.2
	Range (%)	91.5-96.5	98.3-105.1
1:8	Average Recovery (%)	93.8	104.9
	Range (%)	91.7-95.2	101.9-107.6
1:16	Average Recovery (%)	98.9	115.9
	Range (%)	95.0-102.7	113.0-118.7

【Specificity】

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

Human		
BDNF	EGF	FGF basic

【Interfering Substances】

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

Additive	Tolerated concentration
DMSO	5%
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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