EZElisa™ Protein A Residual ELISA Kit

Detection for Residue Used for Natural, Recombinant and Alkali-resisting Protein A

Cat #: A-QEK010

Size: 96 Tests

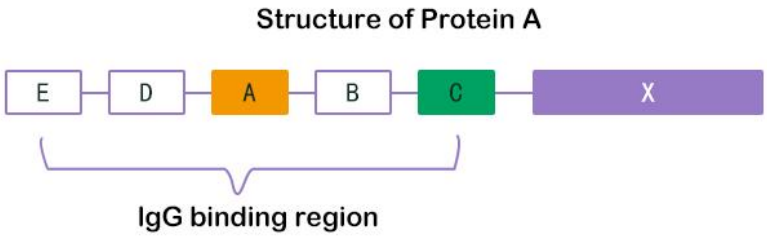
Storage: 2-8 °C in dark (The production date and shelf life are shown on label of the kit.)

Product information

Product Background

Protein A is a kind of protein separated from cell wall of staphylococcus aureus, with molecular weight of 42 kDa. The natural Protein A contains 5 structural domains of E, D, A, B and C that can be bound specifically with Fc segment of antibody IgG molecule, as well as non-Fc binding domain with known functions.

The prepared Protein A affinity chromatography filler is widely applied in the production and purification process of antibody products by covalently coupling Protein A to the purified medium by using the high affinity of Protein A and Fc segment of the antibody.



As a filler of purified column usually used in production of antibody drugs, Protein A may inevitably have Protein A ligand shedding, leading to residue of Protein A in antibody drug. Such minimal residue may have potential hidden dangers for safety (leading to body side effects) and effectiveness (affecting the pharmacodynamics and pharmacokinetics) of the antibody drug.

Requirements for detection of residue of Protein A is specified in “general chapter for recombinant monoclonal antibody products for human use” and the monographs of the two monoclonal antibody drugs “nimotuzumab injection” and “conbercept ophthalmic injection” in *Chinese Pharmacopoeia* Volume III Edition 2020; the requirement for

detection of Protein A residue is also described in American FDA and the United States Pharmacopoeia chapter <130>, which shows that, the detection of Protein A residues in biological products is one of the important indicators of their quality.

Product Purpose

The Protein A residue detection kit (enzyme linked immunosorbent assay) produced by our company is a Protein A residue detection kit with high sensitivity, which can accurately identify several imported and domestic Protein A. It can be used to detect the residue of Protein A in intermediates, semi-finished products and drug products in various biological products in a sensitive, specific and accurate manner. Meanwhile, it can facilitate the Protein A filler manufacturer to monitor the shedding condition of Protein A in specific condition.

The detectable types of Protein A include MabSelect SuRe (Cytiva), MabSelect PrismA (Cytiva), EshmunoA (Merck), TOYOPEARL AF-rProtein A (HC)-650F (Tosoh), recombinant alkaloid-resistant Protein A (NUPTEC), recombinant staphylococcus aureus Protein A (NUPTEC), rProtein A (Chutian Microsphere), etc.

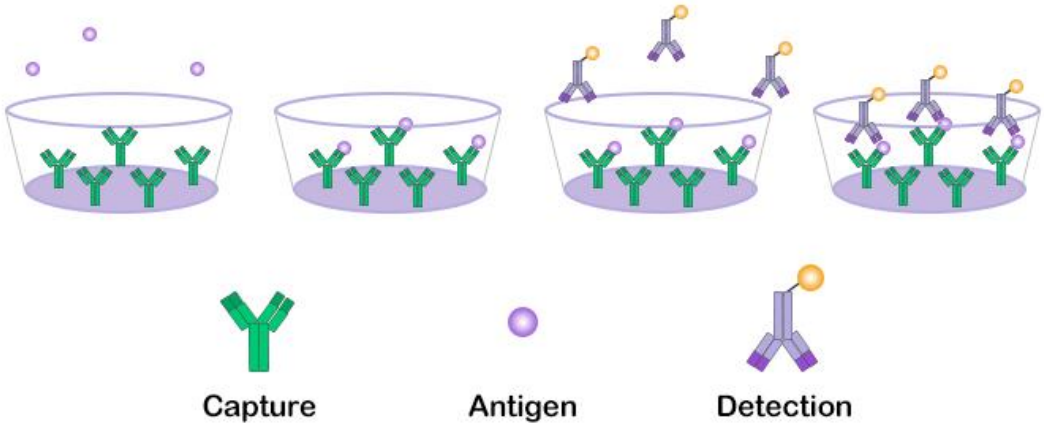
Product Feature

1. Coated with antibodies and detected antibodies are all chicken polyclonal antibodies, which reduces the influence of steric hindrance on the binding of antibody samples to Protein A and ensures the scientific principle.
2. The sample pretreatment method is acid treatment. Direct sample addition reaction after sample dilution, without complicated heating, centrifugation and other steps, the operation is simple.
3. The detection sensitivity is high.

Reaction principles

Double antibody sandwich enzyme-linked immunosorbent assay is used in the kit for determining minimal residue of Protein A in the samples. Prepare a solid phase antibody with a capture antibody-coated 96-well ELISA plate, followed by adding standard and testing the samples, and then add horseradish peroxidase (HRP)-labeled ELISA antibody, to form a solid phase antibody - Protein A - ELISA sandwich conjugate. After the end of reaction, wash it, and then add substrate for chromogenic reaction, and the substrate will change from colorless to blue under the catalysis of HRP and change to yellow finally under the action of stop solution (as shown in the figure). Determine its absorbance value (OD

value) at wavelengths of 450 nm and 630 nm, where, 630 nm is the corrected wavelength. The OD value is positively correlated to content of Protein A in the sample.



Performance and Indicators of Products

Limit of detection: <5.12 pg/mL

Limit of quantification: 5.12 pg/mL

Linearity range: 5.12-500 pg/mL

Accuracy (spiked recovery): 80% - 120%

Accuracy (measured deviation): ≤ 15%

Repeatability (intra-run difference): ≤10%

Materials Supplied and Storage Conditions

Kit components	Size (96 Tests)	Storage conditions
Protein A Clear Microtiter Plate	8 × 12	2~8 °C
Standard (50 ng/mL)	300 µL × 1 tube	2~8 °C
Protein A antibody	15 mL × 1 vial	2~8 °C
Sample diluent(20×)	30 mL × 1 vial	2~8 °C
Washing liquid (20×)	30 mL × 1 vial	2~8 °C
Developing solution A	8 mL × 1 vial	2-8 °C protected from light
Developing solution B	8 mL × 1 vial	2-8 °C protected from light
Stop solution	15 mL × 1 vial	2~8°C
Plate sealer	3	-

The kit shall be stored in dark condition at 2-8 °C. Note that, the opened but not used kit shall still be stored in dark condition at 2-8 °C. The production date and shelf life are shown on label of the kit.

Procedure

Preparation Works

1. Preparation of kit

Place each component of the kit in room temperature for equilibrium for 30 min before starting operation.

2. Consumables and equipment required **(Note: need to be prepared by themselves)**

1) Plate reader, thermostatic oscillator (or thermostatic incubator), plate washer (the plate washing method is hand washing, it may not be used), vortex oscillator, timer

2) High-accuracy pipette and disposable tip (0.5-10 µL, 10-100 µL, 30-300 µL, 100-1000 µL)

3) Deionized water

4) Absorbent paper, EP tube, disposable gloves

3. Reagent preparation **(Note: it shall be prepared as required according to the components provided by the kit)**

1) Sample diluent (1X): add the deionized water into the sample diluent (20X) to dilute for 20 times for standby, for example, add 190 mL of deionized water into 10 mL sample diluent (20X), and mix well.

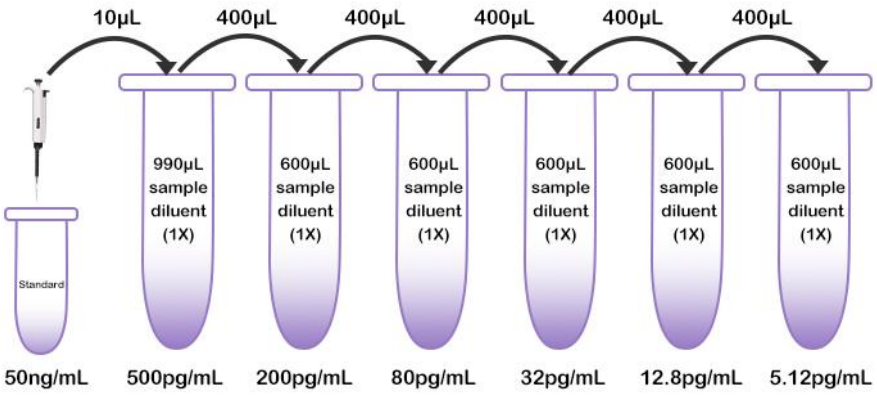
2) Washing liquid (1X): add the deionized water into the washing liquid (20X) to dilute for 20 times for standby, for example, add 190 mL of deionized water into 10 mL washing liquid (20X), and mix well.

(Note: if there are crystals formed in the washing liquid (20×), it shall be placed at room temperature or 37 °C water bath and shaken gently to allow the crystals to dissolve completely before dilution)

3) Preparation of developing solution: mix the developing solution A and developing solution B in the same volume, and protect from light after mixing well. **(Note: it shall not be placed for too long, it shall be prepared 10 min before use. In case that the developing solution becomes blue after mixing, do not use it).**

4. Preparation of standard **(Note: newly-prepared standard solution shall be used for each experiment)**

Dilute the standard (50 ng/mL) with the sample diluent (1X) to 500 pg/mL, and dilute it with serial ratio (the dilution factor is 2.5 times) to prepare the standard, as shown in the figure below:



5. Sample preparation

(1) Sample dilution

Restore the samples to room temperature, mix the sample well; and dilute the samples with the sample diluent (1X). Suitability validation for dilution factor of the sample concentration shall be performed for different samples. The recommended range for sample dilution concentration is 0.01 to 1 mg/mL.

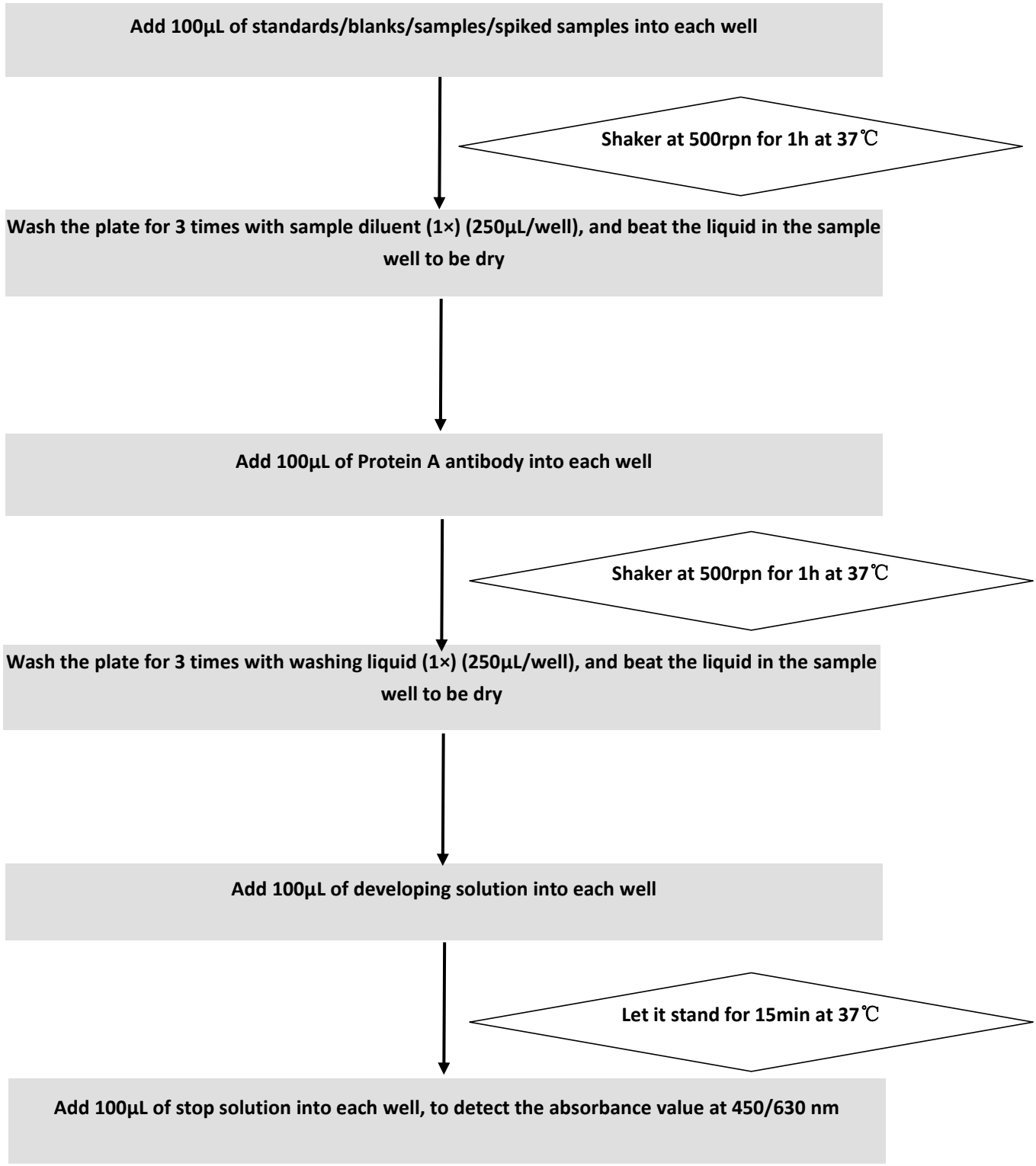
(2) Spiked samples

Select appropriate concentrations of samples to be tested and divide it into 3 to 4 aliquots with the same volume, and add different concentrations of the standard to be tested with the same volume into 2-3 aliquots of the samples, to prepare the samples to be recovered for analysis. The volume added is less than or equal to 10% of the total volume, to prepare 2-3 different addition concentrations of samples to be recovered for analysis, and calculate the concentration of the standard to be added.

Add same volume of sample diluent into another sample, to prepare the base sample. **(Note:It is recommended to prepare more than 50-100 µL for samples and labeled samples)**

Operation Procedures

Operation sketch



Detailed operation steps

All the operations shall be performed at room temperature, and it is recommended that all the spiked wells are subject to replicate-well determination.

1. Restore each component of the kit to room temperature for 30 min, remove the strips required by the test from the aluminium foil bag that has been equilibrated to room temperature, mark the order of the strips with a marker, seal the remaining strips with a plate sealer and place them back into the aluminium foil bag, seal them and store them at 2-8 °C.

(Note: the strips can easily fall off during plate washing, note to make marks.)

2. Sample incubation: add the prepared standards and the samples to be tested into the ELISA plate (the recommended adding sequence: standard well, blank well, sample well, spiked sample well, and the standards shall be added as per the concentration gradient), 100 µL/well, seal the plate with a plate sealer.Cover & incubate on orbital shaker at 500 rpm for 1 hour at 37 °C.

(Note: the sample adding time shall be controlled within 10 min, to avoid shift over time. In case that the plate is not sealed or sealed incompletely during incubation, it may lead to evaporation of reaction liquid, which may lead to experimental error.)

3. Plate washing: carefully uncover the plate sealer after completing incubation, discard the liquid in the well, wash the plate for 3 times (250 µL/well) with the sample diluent (1X), and beat the residual liquid in the well to be dry. (If the plate washing method is hand washing, it can be placed for 1 min after adding the sample diluent (1X); if the plate is washed by the plate washer, vibrate gently for 5 s after adding the sample diluent (1X).)

4. Incubation of Protein A antibody: add Protein A antibody, 100 µL/well, seal the plate with a plate sealer. Cover & incubate on orbital shaker at 500 rpm for 1 hour at 37 °C.

(Note: before adding liquid after each plate washing, the strips shall be checked for fastening, to prevent liquid spillage caused by fastening the strips after adding liquid.)

5. Plate washing: carefully uncover the plate sealer after completing incubation, discard the liquid in the well, wash the plate for 3 times (250 µL/well) with the washing liquid (1X), and beat the residual liquid in the well to be dry. (If the plate washing method is hand washing, it can be placed for 1 min after adding the washing liquid (1X); if the plate is

washed by the plate washer, vibrate gently for 5 s after adding the washing liquid).

6. Color development: add the prepared developing solution into the ELISA plate, 100 µL/well, seal the plate with the plate sealer, protect from light and place at 37 °C for incubation for 20 min.

7. Stopping: add the stop solution, 100 µL/well, reading can be done after the color develops uniformly. **(Note: it is usually completed within 20 min after adding the stop solution).**

8. Reading: place the ELISA plate into the plate reader, set the wavelength as double wavelength of 450/630 nm, and read the absorbance value **(Note: it is recommended to set 5-10 s vibration in the reading procedure of the plate reader)**.

Data Processing

1. Calculation of absorbance value

The calibrated value of light absorbance of each standard or sample is:

OD_{450nm} - OD_{630nm} - absorbance value of the blank control well

2. Plot the standard curve with concentration of the standard as the horizontal coordinate (X) and the calibrated value of light absorption of the standard as the vertical coordinate (Y), and a 4-parameter Logistic mathematical model is recommended to fit the equation:

$$Y = ((A-D) / (1 + (X / C) ^ B)) + D$$

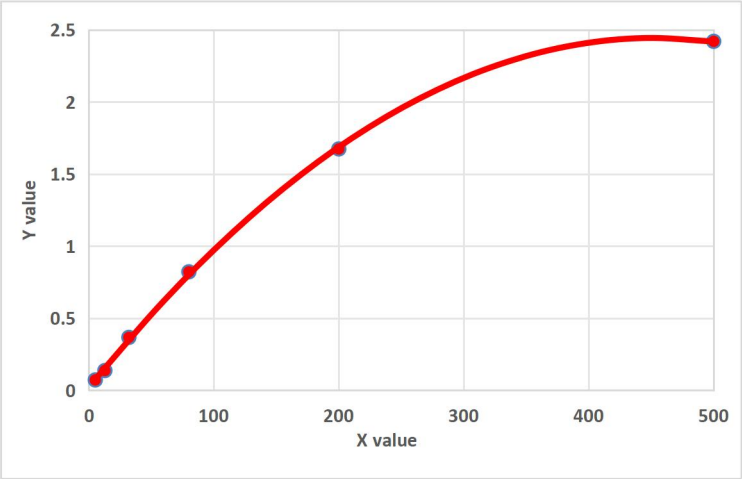
Substitute OD value of the sample (OD_{450nm} - OD_{630nm} - absorbance value of the blank control well) into the formula to calculate the content of Protein A in the sample, and note the dilution factor.

3. If the OD value of the sample to be tested exceeds the highest OD value of the standard curve, the sample shall be redetermined after dilution.

Example presentation:

The following standard curve plot is for reference only, and the standard curve plotted for this experiment standard shall prevail.

Standard curve **pg/mL**	OD value
500	2.4195
200	1.6732
80	0.8210
32	0.3663
12.8	0.1363
5.12	0.0711



Precautions

1. All the components in the kit must be restored to room temperature (20-25 °C) before use.
2. Each component shall be mixed well before use, to guarante uniformness of the reagent, the standards shall be centrifuged for 5 s temporarily, and centralize the liquid on the tube wall and cover onto the bottom of the tube, and place all the reagents back to 2-8 °C immediately after use.
3. The kit must be used within the shelf life, and corresponding standard curve shall be prepared for each test. Mixed use of different batches of related reagents is not recommended.
4. Be careful not to touch the bottom of the microplate during adding liquid to the ELISA plate, to prevent damaging the coating layer. Replace the reagent reservoirs and the tips timely between different samples and steps, to avoid cross contamination.
5. During beating the strips after washing, take care to prevent strips shedding, and the plate sealers shall not be used repeatedly.
6. Black flocs may be generated at high concentration during color development, this is a normal phenomenon, it is minor in degree, it may not affect the final reading results.
7. Note to check whether the correct detection wavelength and fitting equation are selected during reading.
8. The best detection results can only be guaranteed by strictly following the instructions and using all the reagents

supplied with the kit.

9. A general Protein A is adopted in this kit as a standard, and this kit also applies to natural, recombinant and alkali-resistant Protein A from different sources, allowing for direct detection of residue level of Protein A from different sources. In order to further guarantee the accuracy of the results, the Protein A from specific source used actually in the production process can also be used to establish the standard curve on their own.

10. The difference in testing results can be caused by several factors, including operations of the experimenters, using way of the pipettes, plate washing method, reaction time or temperature, storage of the kit etc.

11. Our company is only responsible for the kit itself, but not be responsible for sample consumption caused by using this kit. Please fully consider the possible usage amount of samples before use, and reserve sufficient samples.

12. This kit is only used for in vitro study, not for clinical diagnosis.

Safety Tips

1. All the biological samples have potential biosafety risks, the users shall operate, dispose of and discard the samples in strict accordance with the local laws and relevant provisions.

2. For safety reasons, the operators shall wear the personal protective equipment, such as lab coat, gloves, face masks and safety glasses.

Common Problems and Analysis

If there are problems in the experiment results, please take photos for the color developing results timely, and store the unused strips and reagents properly, and then contact the technical support personnel. You can also refer to the following information to find the reason.

Problem description	**Possible reasons**	**Corresponding countermeasures**
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well
The color development is weak or colorless	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to	Check the liquid aspiration and adding process, and guarantee all

	add the reagent	the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly
		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use
	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution
	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.