Protein A ELISA Kit

Catalog Number: P0111A-96

INTENDED USE

The kit is used for detection of Protein A in intermediates, semi-finished products and drug products in various biological products.

PRINCIPLE

This kit using double antibody sandwich enzyme-linked immunosorbent detection technology to determinate residues 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 . 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.

PRODUCT PERFORMANCE INDICATORS

Detection limit: ≤5.12 pg/mL

Quantitative limit: 5.12 pg/mL

Linear range: 5.12-500 pg/mL

Accuracy (reciprocal recovery rate): 80%-120%

Accuracy (measurement deviation): ≤15%

Repetitiveness (batch difference): ≤10%

MATERIALS PROVIDED

Number	Component	96T	Storage condition
1	Microtiter Plate	8×12	2-8℃
2	Standard1：Applicable to MabSelect SuRe ligand（50 ng/mL）	300 μL×1 tube	2-8℃
3	Standard2：Applicable to MabSelect PrismA ligand（50 ng/mL）	300 μL×1 tube	2-8℃
4	Standard3：Recombinant Protein A（TRUKING MICRO-SPHERE）（50 ng/mL）	300 μL×1 tube	2-8℃
5	Standard4：MaXtar® ARPA ligand ProteinA（Bio-Link）（50 ng/mL）	300 μL×1 tube	2-8℃
6	HRP-Conjugate Antibody	15 mL×1 vial	2-8℃

7	Sample Dilution（20×）	30 mL× 1 vial	2-8℃
8	Wash Buffer (20×)	30 mL× 1 vial	2-8℃
9	Developing Solution A	8 mL× 1 vial	2-8℃ (keep in dark)
10	Developing Solution B	8 mL× 1 vial	2-8℃ (keep in dark)
11	Stop Solution	15 mL× 1 vial	2-8℃
12	Microplate Sealers	3	-

STORAGE

Unopened Kit store at 2-8℃ and the kit is stable for 12 months.

REAGENTS/EQUIPMENTS REQUIRED BUT NOT SUPPLIED

1. Enzyme-labeled instrument, thermostatic oscillator (or thermostatic incubator), plate washer (or hand washing), vortex oscillator, timer
2. Pipettes and pipette tips (0.5- 10 μL, 10- 100 μL, 30-300 μL, 100- 1000 μL)
3. Deionized or distilled water, bibulous paper, EP tubes

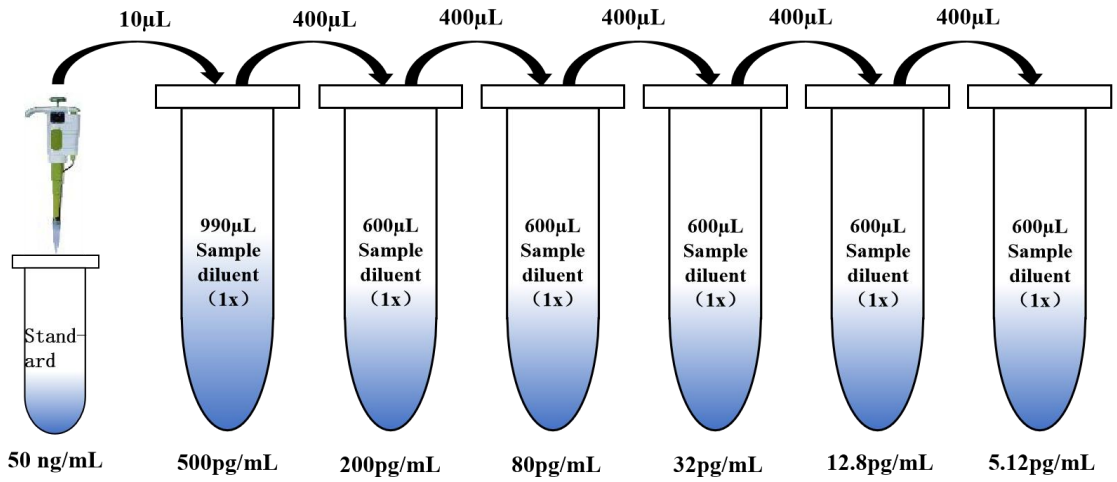
REAGENT PREPARATION

- Sample Dilution (1×): add the deionized water into the Sample Dilution (20×) to dilute for 20 times for standby, for example, add 190 mL of deionized water into 10 mL Sample Dilution(20×), and mix well.
- Wash Buffer (1×): add the deionized water into the Wash Buffer (20×) to dilute for 20 times for standby, for example, add 190 mL of deionized water into 10 mL Wash Buffer (20×), and mix well.If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- Substrate Solution: Mix the developing solution A and developing solution B with equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃.
2. Preparation of the standard curve: dilute the standard (50 ng/mL) with the Sample Dilution(1×) 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:



3. 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, and then shake and incubate at 500 rpm , 37℃ for 1 h.

4. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5 s can be slightly shaken after adding wash buffer).

5. HRP-conjugate antibody incubate: Add 100 μL of HRP-conjugate antibody each well, and then shake and incubate at 500 rpm , 37℃ for 1 h.

6. Washing board: discard the liquid in the well and wash the board with wash buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well.

7. Color development: the pre-prepared substrate solution was added to the enzyme label plate, mixed well (100 μL/well), and incubated in darkness ,37℃ for 15 min.

8. Termination: add stop solution (100 μL /well).

9. Recording: The OD value at 450 nm/630 nm should be measured by placing the plate in the microcoder, and the reading should be completed immediately.

CALCULATION OF RESULTS

1. Calculation of absorbance value: The calibrated value of light absorbance of each standard or sample is: ($OD_{450\ nm}$-$OD_{630\ nm}$)- absorbance valueof the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$) .

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 four -parameter Logistic mathematical model is recommended to fit the equation:Y=（（A-D）/（1+（X/C）^B））+D.Substitute OD value of the sample ($OD_{450\ nm}$ - $OD_{630\ nm}$) - absorbance value of

the blank control well($OD_{450\ nm}$-$OD_{630\ nm}$)) 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.

PRECAUTIONS

1. All the components in the kit must be restored to room temperature (20-25℃) 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℃ 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 study, not for clinical diagnosis.

SAFETY INSTRUCTIONS

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.

PROBLEM ANALYSIS

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
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 add the reagent	Check the liquid aspiration and adding process, and guarantee all 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

The specification is issued in version A/1 with an effective date of 2025/08/13.

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