Version 27-05/24

User's Manual

Protein A ELISA Kit

REF **DEIA-JY2389**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Protein A ELISA kit (enzyme-linked immunosorbent assay) is a highly sensitive kit for detecting Protein A residues. It can accurately identify multiple imported and domestically produced Protein A variants. This kit enables sensitive, specific, and accurate detection of Protein A residues in intermediate, semi-finished, and finished products of various biological products. It also facilitates Protein A affinity resin manufacturers to monitor the shedding of Protein A under specific conditions.

General Description

Product Features:

1. High versatility, suitable for detecting various recombinant and alkali-resistant Protein A variants.
2. The coating antibody and the detection antibody are polyclonal antibodies from chickens, reducing the spatial binding of antibody samples with Protein A.
3. Steric hindrance effects are minimized to ensure scientific validity in principle.
4. The sample pretreatment involves acid treatment, and after sample dilution, direct sample addition reaction without cumbersome steps such as heating or centrifugation, making the operation simple.
5. High sensitivity in detection.

Principles of Testing

The ELISA kit utilizes a double-antibody sandwich enzyme-linked immunosorbent assay technique to detect minuscule traces of Protein A residue in the sample. The capture antibody is immobilized onto a 96-well plate, creating a solid-phase antibody. Then standard samples and test samples are added, and this is followed by the addition of the detection antibody labeled with horseradish peroxidase (HRP), resulting in the formation of a solid-phase antibody-protein A-detection antibody sandwich complex. After incubation the wells are washed, and a substrate is then added to initiate a color reaction. Under the influence of HRP, the substrate transitions from colorless to blue, and finally to yellow upon the addition of the stop solution. The absorbance values (OD values) are measured at 450 nm and 630 nm wavelengths, with 630 nm serving as the reference wavelength.

Reagents And Materials Provided

1. Microplate: 96 well polystyrene microplate (12 strips of 8 wells).
2. Standard 1 MabSelect SuRe (Cytiva): 1×300 µL, 50 ng/mL.
3. Standard 2 MabSelect PrismA (Cytiva): 1×300 µL, 50 ng/mL.
4. Standard 3 Recombinant Protein A: 1×300 µL, 50 ng/mL.
5. Standard 4 MaXtar® ARPA Ligand Protein A: 1×300 µL, 50 ng/mL.
6. Detection Antibody: 1×15 mL, antibody conjugated to horseradish peroxidase (HRP) with preservatives.
7. Sample Dilution 20×: 30 mL of a 20-fold concentrated solution.

8. Wash Buffer 20×: 30 mL of a 20-fold concentrated solution.
9. Chromogen Solution A: 8 mL, avoid direct light.
10. Chromogen Solution B: 8 mL, avoid direct light.
11. 3 Cover foil.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Incubator 37°C
3. Shaker
4. Precision pipettes to deliver 0.5 µl to 1 mL volumes
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare the positive control or sample dilutions

Storage

1. Store all reagents at 2-8°C, and not be frozen or thawed. The product is valid for 12 months.
2. All reagents must be brought to room temperature (20-25°C) prior to use.

Specimen Collection And Preparation

1. Equilibrate the samples to room temperature and mix before loading. If you need to dilute samples or the provided high-concentration standard solution, Buffer (1×) can be used for dilution. For different samples, it is necessary to verify the dilution factor of the sample concentration. The recommended sample concentration range is 0.01-1 mg/mL.
2. Spiked Samples

   2.1 Select the appropriate concentration of the test sample, divide it into 3-4 vials, and add different concentrations of the same volume of standard solution to 2-3 vials to prepare samples for recovery analysis. The concentration of the added standard solution should be less than or equal to 10% of the total volume, creating 2-3 different concentrations for recovery analysis, and calculate the concentration of the added standard solution.

   2.2 Add the same volume of sample diluent (1×) to another part of the sample to prepare the blank sample. (Note: It is recommended to prepare the sample and spiked samples in volumes of 50-100µL.)

Reagent Preparation

1. Wash Buffer (1×): Prepare by Wash Buffer (20×) with deionized water at a 20-fold ratio, for example: mix 10 mL of Buffer (20×) with 190 mL of deionized water. Note: If crystals form in Buffer (20×), gently shake at room temperature or in a 37°C water bath until completely dissolved before dilution. Note: If crystals form in Buffer (20×), gently shake at room temperature or in a 37°C water bath until completely dissolved before

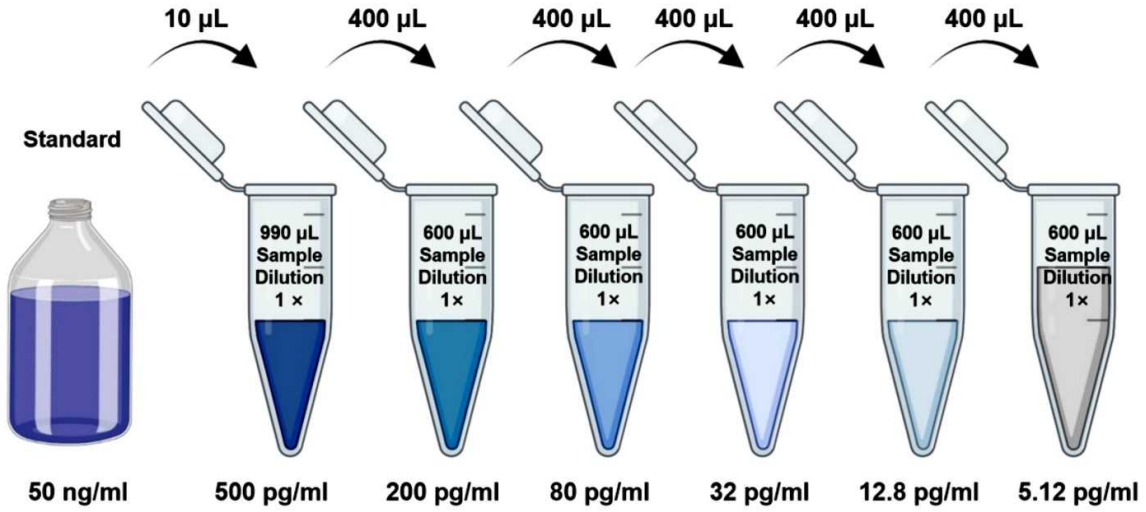
dilution.

2. Sample Dilution Buffer (1×): Prepare by Sample Diluting Buffer (20×) with deionized water at a 20-fold ratio, for example: mix 10 mL of Buffer (20×) with 190 mL of deionized water. Note: If crystals form in Buffer (20×), gently shake at room temperature or in a 37°C water bath until completely dissolved before dilution.
3. Chromogenic Solution: Mix equal volumes of Chromogenic Solution A and Chromogenic Solution B, then store away from light. Note: Do not store for too long; typically, prepare within 10 minutes before use. Do not use if the chromogenic solution turns blue after mixing.
4. Standard Solution: Prepare a new standard solution for each experiment. Different types of Protein A standards are contained in the ELISA kit. Please select the corresponding standard to establish the standard curve based on the type of Protein A affinity resin used.

   4.1 If the corresponding Protein A ligand is not available, for recombinant resins, the standard curve can be established using the recombinant Protein A in this kit;

   4.2 for alkali-resistant resins, the standard curve can be established using the MabSelect SuRe (Cytiva) standard in this kit.

Dilution process: Dilute the standard (50 ng/mL) to 500 pg/mL using the sample diluent (1×), then further prepare the standard by serial dilution (dilution factor: 2.5-fold), as shown in the diagram:



Assay Procedure

1. Allow all components of the ELISA kit to equilibrate at room temperature for 30 minutes, and it is recommended to perform duplicate measurements in all sample wells.
2. Remove the required strips from the aluminum foil bag, and mark the strip with a marker. Note: The strips are prone to detachment during the washing process, so Careful marking is important.
3. Sample incubation: Add the diluted standard and test samples to the plate (recommended: standard well, blank well, sample well and spiked standard well) at 100 µL per well. Seal the plate with a sealing film and incubate at 37°C with 500 rpm shaking for 1 hour. (Note: Control the operating time within 10 minutes to avoid drift over time. Incomplete sealing or lack of sealing during incubation can lead to evaporation of the reaction solution, resulting in experimental errors.)
3. Washing: After incubation, carefully remove the sealing film, discard the liquid from the wells, wash the plate three times with wash solution (1×) at 250 µL per well, and pat dry the residual liquid in the sample wells. (If

washing is done manually, allow the wash solution (1×) to maintain for 1 minute; if using an automated washer, gently shake for 5 seconds after adding the wash solution.)

5. Incubation with Detection Antibody: Add the Detection Antibody to each well at 100 µL per well, seal the plate with a sealing film, and incubate at 37°C with 500 rpm shaking for 1 hour. (Note: Before adding liquid, check whether the strips are secured to prevent liquid splashing caused by strip displacement after liquid addition.)
6. Washing: Same as step 4.
7. Chromogenic Reaction: Add the pre-configured color development solution to the enzyme plate at 100 µL per well, seal the plate with a sealing film, and incubate at 37°C in the dark for 15 minutes.
8. Termination: Add the stop solution at 100 µL per well. Read the absorbance after the color has developed uniformly. (Note: Reading should be completed within 10 minutes after adding the stop solution).
9. Reading: Place the plate into the plate reader, set the wavelength to dual wavelengths at 450/630 nm, and read the absorbance values. (Note: It is recommended to set the plate reader program to include 5-10 seconds of shaking.)

Calculation

1. The calculation of the absorbance correction value for each standard or sample is:

   OD_{450nm} - OD_{630nm} - $OD_{Blank\ Control}$
2. Plot a standard curve with the concentration of the standard as the X-axis and the absorbance correction value of the standard as the Y-axis. It is recommended to use the four-parameter logistic mathematical model fitting equation:

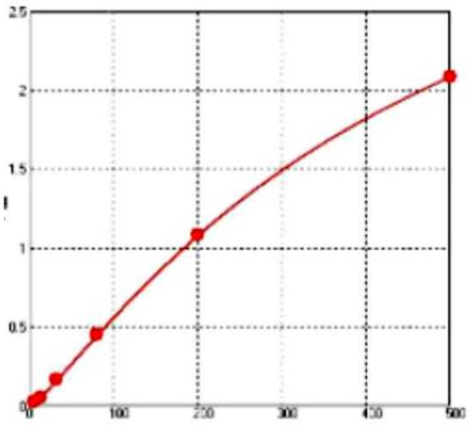
   Y = ((A-D)/(1+(X/C)^B)) + D

Substitute the absorbance correction value of the sample into the formula to calculate the content of Protein A in the sample. (Pay attention to the dilution factor).

3. If the OD value of the sample exceeds the highest OD value on the standard curve, the sample needs to be diluted and retested.
4. Recommended dual-wavelength correction method. Use a wavelength of 630 nm for correction. The corrected OD value is OD_{450} - OD_{630}. This corrected value can be used directly for calculation or, based on data quality, further blank correction can be performed. If a dual-wavelength microplate reader is not available, after reading the OD_{450} data, the data quality should be evaluated before performing blank correction.

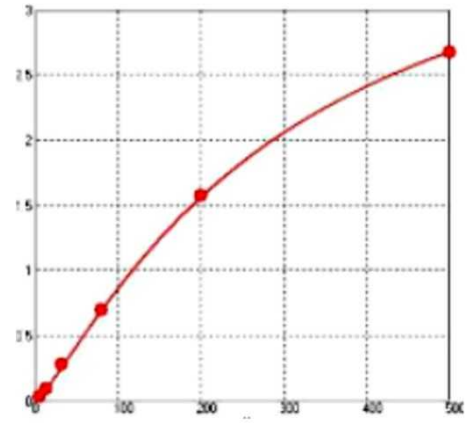
Typical Standard Curve

1. MabSelect SuRe (Cytiva)



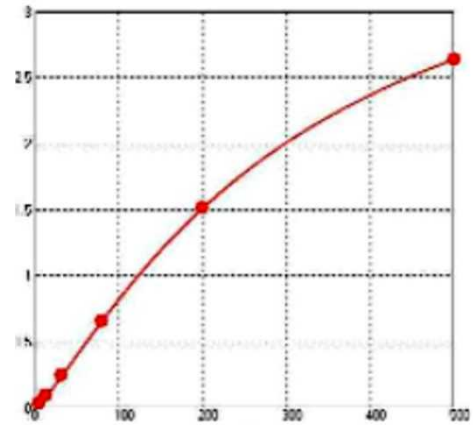
STD (pg/mL)	OD
500	2.0822
200	1.0807
80	0.4478
32	0.1671
12.8	0.0565
5.12	0.0223
500	2.0822

2. MabSelect PrismA (Cytiva)



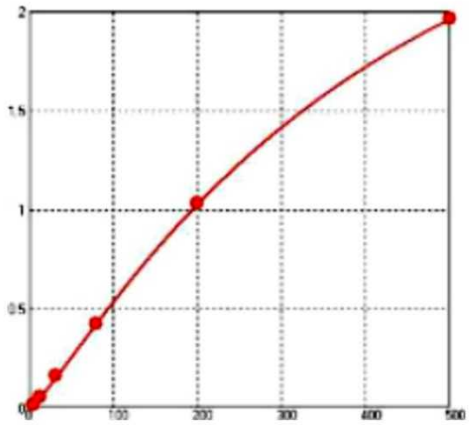
STD (pg/mL)	OD
500	2.6761
200	1.5695
80	0.692
32	0.2735
12.8	0.089
5.12	0.0302
500	2.6761

3. Recombinant Protein A



STD (pg/mL)	OD
500	2.6365
200	1.5137
80	0.655
32	0.2467
12.8	0.0858
5.12	0.027
500	2.6365

4. MaXtar® ARPA Ligand Protein A



STD (pg/mL)	OD
500	1.9603
200	1.0287
80	0.421
32	0.1606
12.8	0.0522
5.12	0.015
500	1.9603

Performance Characteristics

Intra-assay Precision (Precision within an assay): CV% < 10%

Inter-assay Precision (Precision between assays): CV% < 15%

Spike Recovery: 80% - 120%

Detection Range

5.12 - 500 pg/mL

Detection Limit

< 5.12 pg/mL

Sensitivity

5.12 pg/mL

Precautions

1. All components in the kit must be brought to room temperature (20-25°C) before use.
2. Each component must be mixed thoroughly before use to ensure the uniformity of the reagents. The standard needs to be centrifuged briefly for 5 seconds to concentrate all the liquid on the tube wall and cap at the bottom of the tube. Immediately return all reagents to 2-8°C after use.
3. The kit must be used within the validity period, and a corresponding standard curve must be prepared for each test.
4. It is not recommended to use different batches of related reagents in mixed batches.
5. When adding liquid to the microplate, be Careful not to touch the bottom of the microplate to prevent damage to the coating layer.
6. Change the sampling tank and tip in time between the same sample and steps to avoid cross-contamination.

7. When patting the slats dry after washing, be Careful to prevent the slats from falling off, and be Careful not to reuse the sealing film.
8. High concentrations may produce black floc during color development, which is a normal phenomenon and will not affect the final reading result if it is slight.
9. When reading, pay attention to check whether the detection wavelength and fitting equation are selected correctly.
10. Only by strictly abiding by the instructions and using all the reagents provided by this kit can the best detection results be ensured.
11. This kit is also suitable for recombinant and alkali-resistant protein A from different sources, and can directly detect the residual levels of protein A from different sources. In order to further ensure the accuracy of the results, a standard curve can be established by oneself using specific sources of protein A actually used in the production process.
12. Different test results can be caused by various factors, including the operator's technique, pipetting, plate washing technique, reaction time or temperature, and the storage of the reagent kit.
13. This ELISA kit is for in vitro research use only and is not intended for clinical diagnosis.

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