Version 30-04/25

User's Manual

Recombinant Protein A ELISA Kit

REF **DEIA-BY020**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This product is a universal detection kit used for quantitative detection of residue natural or recombinant Protein A concentration in biopharmaceutical samples.

General Description

Protein A affinity chromatography resin is a commonly used tool for separating and purifying antibodies in the production and purification process of antibody products. However, during the antibody purification process, Protein A may leach, resulting in the presence of Protein A in the antibody products, which affects the purity and potency of the products and brings significant problems to the pharmaceutical industry.

Protein A includes natural Protein A, recombinant Protein A, and alkali resistant recombinant Protein A with significantly different structures from natural Protein A, such as GE's MabSelect SuReTM Protein A.

Principles of Testing

This kit is based on a sandwich ELISA. Protein A present in the test sample and calibrators is captured by Protein A antibody that has been pre-adsorbed on the surface of microtiter wells, forming an immune complex. After sample binding, unbound proteins and molecules are washed off, and HRP-labeled detection antibody is added to the wells to bind to the Protein A in the calibrators or samples, forming an immune complex. The unbound components are washed away, and a color substrate is added. The colorimetric reaction produces a blue product, which turns yellow when the reaction is terminated by addition of stop solution. The OD value is measured at 450 nm. The OD450 nm is proportional to the amount of Protein A analyte present in the sample and a standard curve can be generated. The quantity of Protein A in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Reagents And Materials Provided

1. Protein A MicroPlate: 12×8; Store at 2-8°C in sealed foil bag with desiccant.
2. Recombinant Protein A Standard: 3 vials, lyophilized; Store at 2-8°C.
3. Protein A 100× HRP-Antibody: 2×30 µL; Store at 2-8°C.
4. Assay Diluent: 2×25 mL; Store at 2-8°C.
5. 20× Wash Buffer Concentrate: 30 mL; Store at 2-8°C.
6. Substrate Solution: 12 mL; Store at 2-8°C in the dark.
7. Stop Solution: 12 mL; Store at 2-8°C.
8. Adhesive plate cover: 3 sheets.

Materials Required But Not Supplied

1. Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions

2. Test tubes
3. Distilled or Deionized H_2O
4. Microtitre Plate reader
5. Assorted glassware for the preparation of reagents and buffer solutions
6. Centrifuge for sample collection

Storage

Stored at 2-8°C until the expiration date.

Specimen Collection And Preparation

1. The sample should be clarified and the insoluble precipitate should be removed by centrifugation.
2. Appropriate dilution can be made according to the actual sample situation (it is recommended to complete the applicability study and determine the sample dilution ratio for the first use).
3. The Protein A remaining in the sample generally binds to the antibody, interfering with the detection results, so the Protein A in the sample must be completely dissociated from the antibody for accurate detection. Heating treatment can effectively separate Protein A and antibodies, with the antibodies denaturing and precipitating upon heating, then being removed by centrifugation, while Protein A remains in the supernatant. A higher concentration of antibodies in the sample will interfere with the accuracy of the detection, The Protein A remaining in the sample generally binds to the antibody, interfering with the detection results, so the Protein A in the sample must be completely dissociated from the antibody for accurate detection. Heating treatment can effectively separate Protein A from the antibody, with the antibody denaturing and precipitating upon heating, then being removed by centrifugation, while Protein A remains in the supernatant. A higher concentration of antibodies in the sample will interfere with the accuracy of the detection, so the antibodies need to be diluted to below 10 mg/mL.

Method

1) Dilute 8 standards (10000.0, 3333.3, 1111.1, 370.4, 123.5, 41.2, 13.7, and 0 pg/mL)(see 'Reagent Preparation') as needed, with at least 300 µL prepared for each concentration, and place in a 1 mL centrifuge tubes for later use;
2) Dilute the samples with Assay Diluent to a protein concentration below 10 mg/mL as needed, with at least 300 µL prepared for each sample, and place in a 1 mL centrifuge tubes for later use;
3) Incubate the prepared standards and samples in a boiling water bath or a 100°C metal bath for 10 minutes;
4) Allow the treated standards and samples to stand at 20-25°C for 5-10 minutes (to return to room temperature);
5) Centrifuge the standards and samples at 13000rpm for 5 minutes;
6) Transfer the supernatant containing Protein A to a new centrifuge tube for later use.

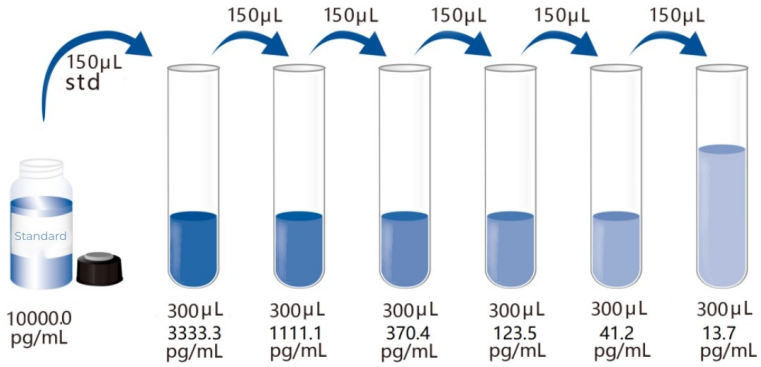
Reagent Preparation

Bring all reagents to room temperature (16°C to 25°C) before use.

1. **20× Wash Buffer Concentrate** - The Wash Buffer supplied is a 20× Concentrate and must be diluted 1/20

with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). And put the unused part back to the refrigerator.

2. **Recombinant Protein A Standard**: Add Assay Diluent to the lyophilized Recombinant Protein A Standard and dilute Recombinant Protein A Standard to 10000.0 pg/mL, left for 15 minutes, and gently mixed after it was fully dissolved. Then perform a 1:3 dilution (standard curve concentration: 10000.0, 3333.3, 1111.1, 370.4, 123.5, 41.2, 13.7 and 0 pg/mL).



Note:: If the redissolved original standard (10000.0 pg/mL) is not used up, please divide it and store it in a refrigerator below -18 °C for two months. The diluted standard should be discarded.

3. **Protein A 100× HRP-Antibody**: Calculate the required amount of working conjugate solution for each microtitre plate test strip. Dilute Protein A 100× HRP-Antibody 100 times with Assay Diluent to prepare a working solution. Prepare 30 minutes before use for daily use only.

Assay Procedure

1. Determine the number of strips required. Leave these strips in the plate frame. Place unused strips in the foil pouch with desiccant and seal tightly. Store unused strips at 2-8°C.
2. Add 50 µl of appropriately diluted standards or samples to each well. Run each standard, sample, or blank in duplicate. Carefully cover the wells with a new adhesive plate cover and incubate for one (1) hour on plate shaker (500rpm) at room temperature, 20-25°C.
3. Empty the contents of the wells and wash by adding 350 µL of 1× Wash Buffer to every well. Repeat the wash 5 times. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
4. Except for the blank wells, add 50 µl of Protein A 1× HRP-Antibody to each well. Carefully cover the wells with a new adhesive plate cover and incubate for one (1) hour on plate shaker (500rpm) at room temperature, 20-25°C.
5. Repeat step 3.
6. Add 100 µl of Substrate Solution into each well and allow the enzymatic reaction to develop a blue color at room temperature (20-25°C) in the dark for 15 minutes.
7. Stop the reaction by adding 100 µl of Stop Solution to each well. Tap plate gently to mix. Determine the absorbance (450 nm) of the contents of each well within 10 minutes.

Calculation

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average Blank OD from the average OD for each standard and sample.

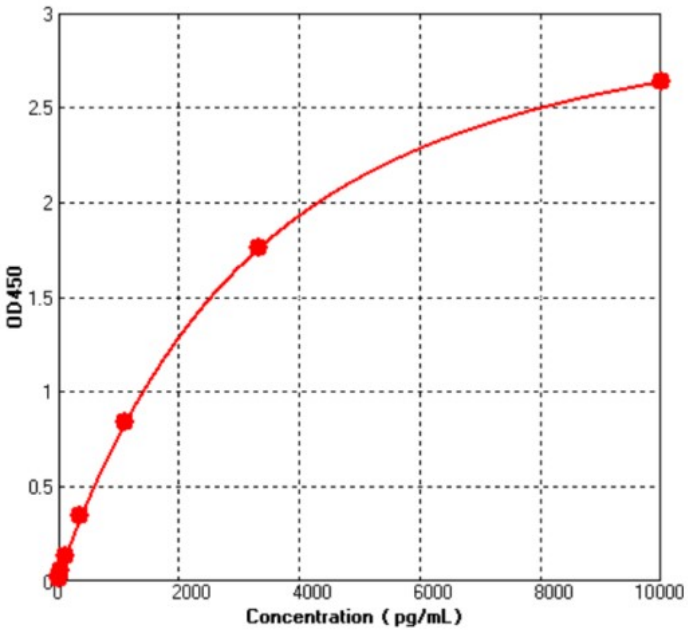
   Average Net OD = Average OD - Average Blank OD

2. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding Protein A standard concentration on the horizontal (X) axis using curve-fitting software. (A four or five parameter logistic curve fit is recommended)
3. Determine the amount of Recombinant Protein A in each unknown sample by noting the Recombinant Protein A concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample. If the OD value of the sample is higher than the upper limit of the calibration curve, it should be diluted and retested. Multiply the observed Protein A concentration by the dilution factor to determine the concentration of Recombinant Protein A in the original, undiluted sample.

Typical Standard Curve

This standard curve is for demonstration only. A standard curve must be generated for each assay.

Standard	$OD_{450-630}$				Conc. (pg/mL)			
(pg/mL)	1	2	3	Average	1	2	3	Average
10000.0	2.657	2.653	2.602	2.635	10409.3	10332.1	9435.1	10058.8
3333.3	1.769	1.804	1.754	1.764	3353.4	3480.1	3300.6	3378.0
1111.1	0.867	0.802	0.839	0.836	1158.9	1049.1	1111.1	1106.4
370.4	0.349	0.341	0.355	0.346	383.8	373.5	391.6	383.0
123.5	0.132	0.127	0.128	0.129	120.7	115.1	116.2	117.3
41.2	0.054	0.052	0.055	0.054	37.9	35.9	38.9	37.6
13.7	0.029	0.028	0.030	0.029	14.1	13.2	15.0	14.1
0.0	0.013	0.015	0.017	0.015	0.9	2.3	3.9	2.4
r^2	0.99999							



Precision

Intra-assay Precision (Precision within an assay): ≤20%. Inter-assay Precision (Precision between assays) : ≤20%.

Sensitivity

LOD: 7 pg/mL

LOQ: 13.7 pg/mL

Precautions

1. Please store the reagent kit at 2-8°C before use. Except for the redissolved Alkaline-Resistant Protein A Standard, other components should not be frozen;
2. The volume of Protein A 100× HRP-Antibody is small, and during transportation, shaking and possible inversion may cause the liquid to adhere to the tube walls or bottle caps. Therefore, please centrifuge briefly before use to allow the liquid adhering to the tube walls or bottle caps to settle to the bottom of the tube;
3. 20× Wash Buffer Concentrate taken out from the refrigerator may have crystals, which is a normal phenomenon. Warming of the concentrate to 30-35°C before dilution can dissolve crystals;
4. Avoid mixing components from different batch numbers of reagent kits;
5. Pay attention to fully mix the solution to ensure that the liquid added to the wells is uniform;
6. For research use only. Not for diagnostic purposes. In vitro use only.