EIV32025E

ExCell Bio

ResiQuant® Alkali-Resistant Protein A ELISA Kit 2G

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	CRP00-3031S
	CRP00-3031
	CRP00-3032

PRODUCT DESCRIPTION

Protein A affinity chromatography resin is commonly utilized for purification of antibody-based products. However, Protein A may be disassociated from the spherical polymer beads and co-eluted with antibodies during the purification process, leading to contamination of the final product with Protein A. This impurity poses significant challenges to the pharmaceutical industry as it can negatively impact the purity and efficacy of the product. For instance, the Chinese Pharmacopoeia (ChP) (2020) mandates that the residual amount of Protein A in Lintuzumab injection, as detected by enzyme-linked immunosorbent assay (ELISA), should not exceed 0.001% of the total protein content. Commercially available Protein A exists variant forms like recombinant nature form, and artificially modified alkali-resistant form, such as MabSelect SuRe™ Protein A, which is structurally different from the natural Protein A. ResiQuant® CRP00-303* kits are specifically designed for highly sensitive detection of alkali-resistant Protein A, with an ultra-low limit of quantitation at 4.9 pg/mL.

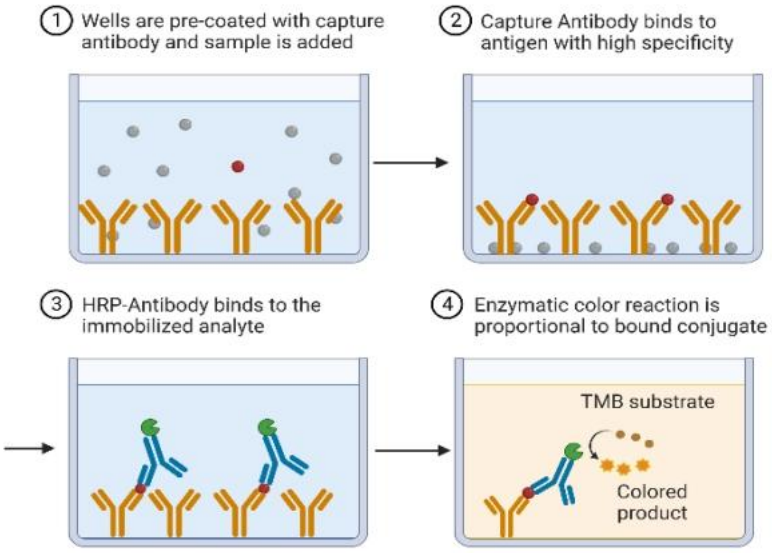
For the first time, it is recommended to conduct a suitability study to evaluate the product's compatibility with the sample matrix and establish the appropriate dilution conditions for test.

PRINCIPLES OF THE TEST

The assay employs a sandwich immunoassay methodology for the quantitation of alkali-resistant Protein A. Microplates are coated with antibodies that exhibit specificity for alkali-resistant Protein A. Subsequently, the standards or test samples are pipetted into the microplate wells, facilitating the formation of immune complexes between the immobilized antibodies and the Protein A present in the samples. After a series of washes to remove unbound components, the antibodies conjugated with Horseradish peroxidase (HRP) are added to selectively bind with Protein A within the immune complexes. Following a further round of washes to eliminate unbound HRP-Antibody conjugates, the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB) is introduced, initiating an enzymatic reaction. This reaction involves HRP-mediated oxidation of TMB in the presence of hydrogen peroxide (H_2O_2), yielding a blue-colored product that exhibits maximum absorbance at 655 nm. The enzymatic reaction is subsequently halted by the addition of a stop solution, which turns the color to yellow with peak absorbance at 450 nm. The optical density (OD) value at 450 nm, measured by an enzyme-linked immunosorbent assay (ELISA) reader, is proportional to the concentration of Protein A within the samples.

Quantitative determination of Protein A is achieved with a simultaneously prepared standard curve, using the standard supplied within the kit.

Schematic diagram:



PRODUCT PERFORMANCE

1. Sensitivity: The limit of detection (LOD) for alkali-resistant Protein A is 2.5pg/mL, and the low limit of quantitation (LLOQ) is 4.9 pg/mL.
2. Precision: The coefficient of variation (CV) within the quantitation range is no more than 20% for both intra- and inter-assay.

PRODUCT APPLICATION

This product is a generic assay kit designed for the quantitative detection of alkali-resistant Protein A impurities in samples.

PRODUCT SPECIFICATIONS

Catalog Number	Name	Size
CRP00-3031S	ResiQuant® Alkali-Resistant Protein A ELISA Kit 2G	48T
CRP00-3031	ResiQuant® Alkali-Resistant Protein A ELISA Kit 2G	48T
CRP00-3032	ResiQuant® Alkali-Resistant Protein A ELISA Kit 2G	96T

REAGENTS PROVIDED

Name	96 Tests	48 Tests	Store
Alkali-Resistant Protein A Standard	2	1	2℃ to 8℃
Protein A Microplate	8× 12	8× 6	2℃ to 8℃
Protein A 100× HRP-Antibody	60 µL	30 µL	2℃ to 8℃
Protein A Assay Diluent	2× 25 mL	25 mL	2℃ to 8℃
20× Wash Buffer Concentrate	30 mL	30 mL	2℃ to 8℃
Substrate Solution	12 mL	6 mL	2℃ to 8℃ (Light-Sensitive)
Stop Solution	12 mL	12 mL	2℃ to 8℃
Plate Sealer	3	2	/

INSTRUCTION FOR USE

I. Materials required but not provided

1. Microplate reader capable of measuring absorbance at 450 nm (If wavelength correction is needed, additional wavelength at 630 nm or 570 nm is required).
2. 0.5-10 µL, 2-20 µL, 20-200 µL, 100-1000 µL adjustable micropipettes with disposable tips.
3. Microplate Shaker.
4. Deionized water.

II. Sample collection

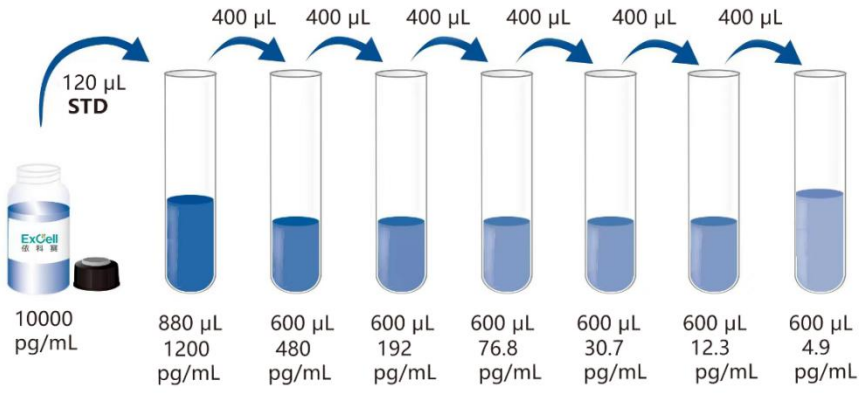
1. Samples should be clarified and any precipitate should be removed by centrifugation.
2. The sample should be diluted appropriately with Protein A Assay Diluent according to the pre-determined

condition (It is recommended to complete the applicability study to confirm the appropriate dilution conditions for sample detection).

III. Preparation of reagents

1. It is recommended to remove the assay kit from the refrigerator 20 minutes in advance to allow it to equilibrate to room temperature.
2. Dilute 20× Wash Buffer Concentrate with deionized or distilled water to prepare the wash buffer. Store the unused solution at 2℃ to 8℃.
3. Reconstitute the Protein A standard with Protein A Assay Diluent and dilute it to a concentration of 10000 pg/mL. Ensure thoroughly mixing to achieve complete reconstitution and allow the standard to incubate for 15 minutes. Then, dilute it to a concentration of 1200 pg/mL as the initial dilution step. Prepare an eight-point standard curve using 2.5-fold serial dilutions with the assay diluent. The assay diluent itself serves as the zero standard (0 pg/mL). The recommended concentrations for the standard curve are as follows: 1200, 480, 192, 76.8, 30.7, 12.3, 4.9 and 0 pg/mL.

The dilution of standard solution:



Note: *Please aliquot the reconstituted stock solution (10000 pg/mL) and store the unused stock solution at -18℃ or lower in two months. Use freshly prepared diluted standards every time.*

4. HRP-Antibody working solution: Freshly prepare everytime the working solution by diluting 100 × HRP-Antibody 100-fold with Protein A Assay Diluent in an amount sufficient advance 30 minutes for the current experiment.
5. Sample handling:The residual Protein A in the sample usually binds to the antibody and interferes the test. Therefore, the Protein A in the sample must be completely dissociated from the antibody for accurate detection. Heat treatment can effectively separate Protein A from antibody, the antibody is denatured and

precipitated by heating, and then removed by centrifugation. Protein A is left in the supernatant. High level of the antibody in the sample often interferes with the assay, proper dilution for the test samples is recommend according to the minimum required dilution (MRD).

Sample handling details:

1. According to the amount required, it is recommended to prepare at least 400 µL for each concentration (1200, 480, 192, 76.8, 30.7, 12.3, 4.9 and 0 pg/mL).
2. According to the amount required, properly dilute all samples based on the MRD with Protein A Assay Diluent, it is recommended to prepare at least 400 µL for each sample.
3. Incubate samples, standards for 10 minutes in a boiling water bath or a block heater.
4. Allow the samples and standards cooling for 5-10 minutes to equilibrate to room temperature.
5. Centrifuge samples and standards for 5 minutes at 13000 rpm, room temperature.
6. Transfer the supernatants to new tubes.

IV. Washing

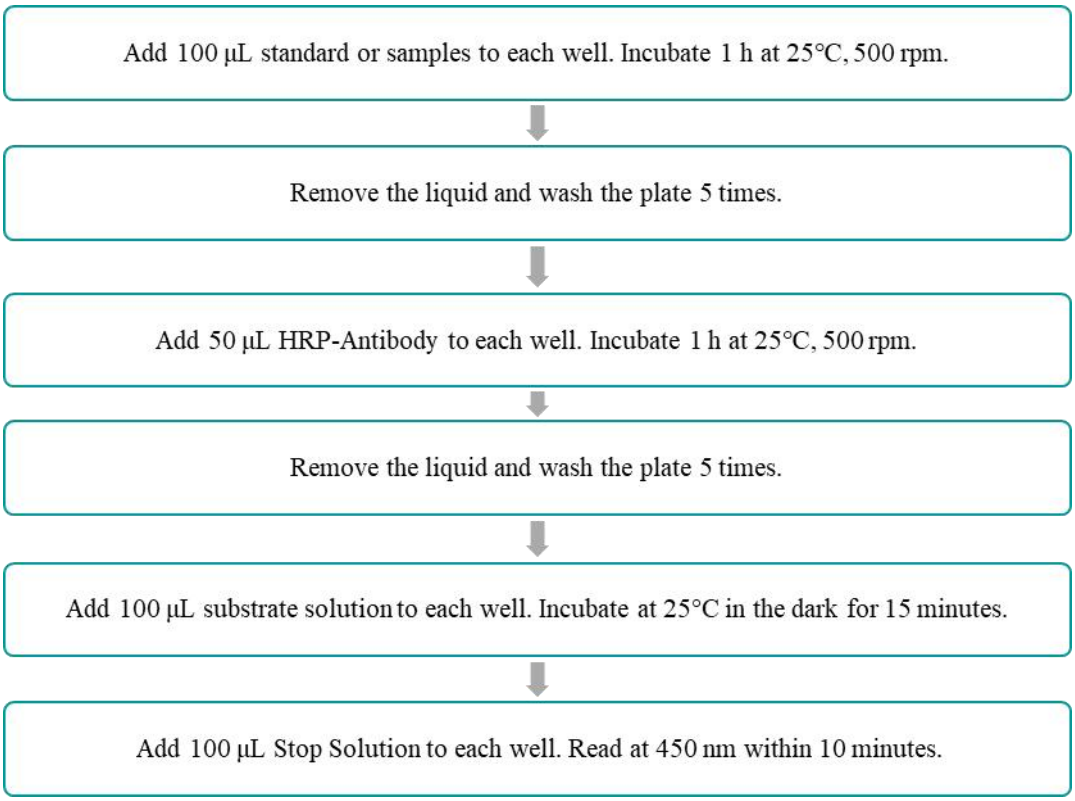
1. Hand-wash: add 300 µL of wash buffer into each well, allow it to sit for 10 seconds, then pour off the liquid in the well. Invert the plate and blot it against clean paper towels. Wash the plate 5 times.
2. Automatic washing machine: set the volume to 300 µL, and the interval between aspiration and dispensing is 10 seconds. Wash the plate 5 times.

V. Assay procedure

1. Bring all reagents and samples to room temperature before use. Remove excess microplate strips from the plate frame, return them to the foil pouch containing a desiccant pack, reseal tightly and store at 2℃ to 8℃.
2. Set the blank well (If the plate is measured with dual-wavelength, the blank may not be necessary).
3. Prepare samples and standards in advance.
4. Add **100 µL** per well of either standards or samples. Seal the wells with the plate sealer. Incubate the plate at **25℃** and shake for **60 minutes,** using a microplate shaker (500 rpm).
5. Prepare HRP-antibody working solution in advance.
6. Remove the liquid from the wells and wash the plate 5 times.
7. Add **50 µL** of biotinylated antibody working solution to each well. Seal the wells with the plate sealer. Incubate at **25℃** and shake for **60 minutes**, using a microplate shaker (500 rpm).
8. Remove the liquid from the wells and wash the plate 5 times.

9. Add **100 µL** of Substrate Solution to the wells, Incubate at **25℃** in the dark for **15 minutes**.
10. Add **100 µL** of Stop Solution to the wells. Read the optical density (OD) of each well within 10 minutes using a microplate reader set to 450 nm. If wavelength correction is available, the OD value at additional wavelength (630 nm or 570 nm) should be collected. For single wavelength mode, the OD value of blank well shall be subtracted from that of each standard or sample.

VI. Assay Procedure Summary

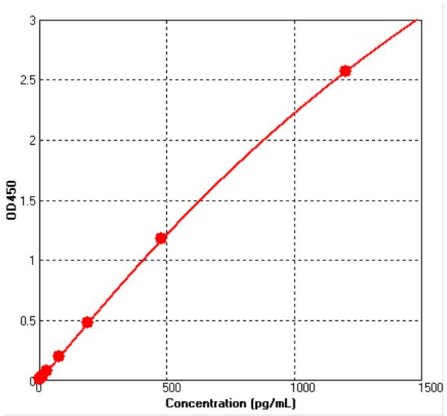


ANALYSES OF RESULTS

1. Create a standard curve by plotting the mean optical density (OD) of each standard concentration as the y-axis against the standard concentration on the x-axis. It is recommended to use a four-parameter fitting equation for the standard curve. Calculate the concentration of samples based on their OD values.
2. The coefficient of determination (R^2) for the standard curve should be no less than 0.99. Discard those abnormal data, the deviation between the back calculated and the theoretical concentration of each standard should be within ±20% (±25% for the upper or lower limits of quantitation).
3. If the OD value of the sample exceeds the high end of the standard curve, the sample should be appropriately diluted before further measurement. Be aware that the dilution factor should be counted with back calculated concentration together.
4. The concentration of samples should be calculated with the standard curve in the parallel assay.

Representative Standard Curve:

Standard (pg/mL)	**$OD_{450-630}$**			**Concentration (pg/mL)**		
	1	2	3	1	2	3
1200	2.647	2.515	2.552	1246.4	1165.9	1188.2
480	1.203	1.148	1.179	492.3	468.0	480.2
192	0.470	0.476	0.496	186.0	188.4	196.3
76.8	0.198	0.192	0.206	78.1	75.7	81.3
30.7	0.089	0.076	0.081	34.1	28.8	30.8
12.3	0.039	0.039	0.034	13.0	13.0	10.9
4.9	0.020	0.021	0.021	4.5	5.0	5.0
0	0.008	0.012	0.009	----	----	----
R^2	0.99999					



Note: *This graph is provided for reference purpose only. Data analysis was using ELISA Calc software with a four-parameter fitting model.*

NOTES

1. Store the reagents at 2℃ to 8℃. The reconstituted standard stock solution should be kept at -18℃ or below.
2. The concentrated HRP-Antibody is supplied in a small volume. Turbulence or inversion happened during transportation may cause spray of the solution elsewhere on the wall or the cap. Therefore, please spin the tubes shortly before use to harvest all liquid to the bottom of the tube.
3. In case crystal sediment appears in the 20× Wash Buffer Concentrate, warm it up at 37°C to dissolve the sediment before use.
4. Do not mix or substitute reagents with those from other lots or different kits.
5. Ensure thoroughly mixing in preparing the solutions to ensure the reactions are consistent.
6. It is recommended to test all samples and standards in duplicate form at least.

DISCLAIMER

1. The product should be used according to the instructions in the manual. If the experimenter fails to operate according to the instructions, our company will not be responsible for any deviation in product performance caused by this.
2. The product is only used for scientific research and commercial production, and is not suitable for clinical diagnosis and treatment. Otherwise, all consequences arising shall be borne by the experimenter, and our company shall not be responsible.