resDetect™ Universal Protein A Quick ELISA Kit (Boiling-free)

Cat. No. RES-A029 **Size: 96 tests**

Intended Use

The resDetect™ Universal Protein A Quick ELISA Kit (Boiling-free) is a complete kit for the quantitative determination of recombinant Protein A and various unnatural Protein A constructs such as alkaline-resistant Protein A, etc. It is compatible to almost all protein A resins with different Protein A variants in the market. The resDetect™ Universal Protein A Quick ELISA Kit (Boiling-free) provides an effective sample treatment method to dissociate Protein A from IgG samples without boiling and centrifugation step. These solve the previous problem of poor recovery in some sample detection. At the same time, the procedure has been simplified with over 1 hour eliminated from sample treatment time.

The kit is for research and manufacturing use only and is not intended for diagnostic use in humans or animals.

Kit Characteristics

- Universality - Suitable for detection of natural or structurally conserved recombinant forms of Protein A and alkaline-resistant Protein A variants, such as MabSelect SuRe™ and other ligands
- Fast time to results - less than 2 hours
- Accuracy - Tracebility of Protein A standards against BSA Standard with validated pharmacopoeia quantitation methodology
- Extensive validation - Validation Report (ICH compliant) available on request
- High sensitivity - Sensitivity < 40 pg/mL of recombinant Protein A and MabSelect™ SuRe Protein A or other Protein A ligands
- High IgG tolerance - Accurately quantify protein A in up to 20 mg/mL antibody
- Excellent buffer compatibility

Background

Protein A is a cell wall protein of Staphylococcus aureus, it has a variety of specific biological characteristics. Due to its high affinity with the Fc part of certain immunoglobulins (especially IgG), it is widely used in the purification of biopharmaceuticals (such as antibodies, vaccines, etc.). However, during the purification, protein A may leach from the purification column and result in contamination of the antibody drugs prepared. Once the remaining protein A enters the human body, it will easily activate the immune response of the organism, and there is a safety risk, so there are strict regulations on the residual level of Protein A in antibody drug preparations. Therefore, the detection of residual Protein A in antibody drugs purified from Protein A purification column is a key quality control step in the production process of antibody drug preparations.

The resDetect™ Universal Protein A Quick ELISA Kit (Boiling-free) can detect protein A or unnatural protein A variants within 2 hours, it is high sensitive and easy to operate because the sample processing has been changed from the original boiling method to adding denaturing reagents. The sample processing process has been shortened by one hour. Whether in upstream small-scale trials or downstream large-scale of antibody production processes, this kit can help you to accurate analysis of samples, monitor the protein A levels and ensure product quality.

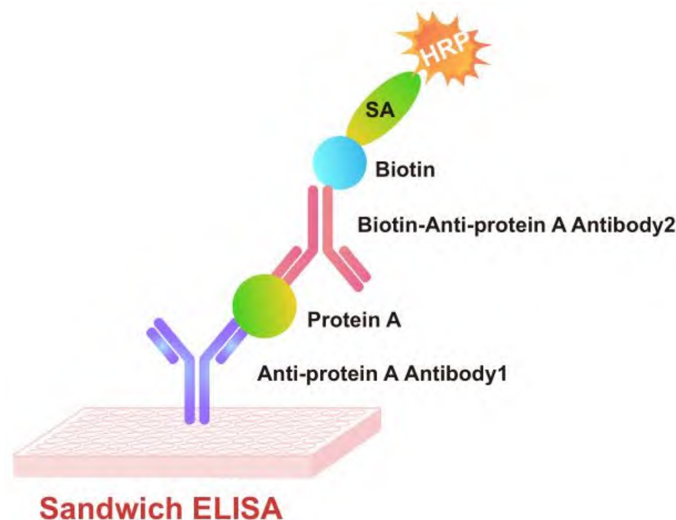
Applications

The kit is developed for the detection of natural or structurally conserved recombinant forms of Protein A and

alkaline-resistant Protein A variants, such as MabSelect SuRe™ , MaXtar® ARPA ligand (Bio-Link Co.), UniMab® 50 Protein A (NanoMicro), MabSelect PrismA™ etc. in bioprocess manufacturing applications. It is used as a universal protein A and variants ligand detection tool to aid in optimal antibody purification process development and in routine quality control of in-process streams as well as final product.

Principle of the Procedure

The resDetect™ Universal Protein A Quick ELISA Kit (Boiling-free) is used to measure the levels of protein A and protein A variants by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with anti-protein A polyclonal antibody. Firstly, the standard samples provided in kit and your samples are treated with Denaturation Buffer to dissociation of protein A and antibody, stand a few minute. Before adding standards and samples, add the Biotin-Anti-Protein A Antibody to the plate to ensure that the standard samples are neutralized by the Biotin-Anti-Protein A Antibody buffer solution and protect the pre-coated antibody on the plate. Then, add the standard samples and your samples to the plate and form Antibody-antigen (Protein A) - biotinylated antibody complex, incubate and wash the wells. Next add Horseradish peroxidase conjugated streptavidin (Streptavidin-HRP) to the plate, incubate and wash the wells to remove any unbound reactants. At last, load the tetramethylbenzidine (TMB) substrate into the wells and monitor a blue color. The reaction is stopped by the addition of a stop solution and the color turns yellow. The intensity of the absorbance can be measured at 450nm and 630nm on a microtiter plate reader. The OD Value reflects the amount of protein A.



If you have any questions, please contact our technical support team at: TechSupport@acrobiosystems.com
http://www.acrobiosystems.com

Reagents & Materials Provided

Catalog	Components	Size (96 tests)	Storage
RES029-C01	Pre-Coated Anti-Protein A Antibody Microplate	1plate	2-8℃
RES029-C02A	Alkali-Tolerant Recombinant Protein A Standard (1μg/mL)	100μL	2-8℃
RES029-C02B	MaXtar® ARPA ligand Protein A Standard (Bio-Link Co.) (1μg/mL)	100μL	2-8℃
RES029-C02C	Recombinant PrismA Standard (1μg/mL)	100μL	2-8℃
RES029-C03	Recombinant Protein A Standard (1μg/mL)	100μL	2-8℃
RES029-C04	Biotin-Anti-Protein A Antibody	1.5mL	2-8℃
RES029-C05	Streptavidin-HRP	10μg	2-8℃, avoid light
RES029-C06	10×Sample Dilution Buffer	15mL	2-8℃
RES029-C07	Denaturation Buffer	15mL	2-8℃
RES029-C08	20×Washing Buffer	30mL	2-8℃
RES029-C09	Antibody Dilution Buffer	15mL	2-8℃
RES029-C10	Streptavidin-HRP Dilution Buffer	15mL	2-8℃
RES029-C11	Substrate Solution	12mL	2-8℃, avoid light
RES029-C12	Stop Solution	8mL	2-8℃

Materials & Equipment Required But Not Provided

Items	Specifications
Single or multi-channel micropipettes	Need to meet 10 μL, 300 μL, 1000 μL injection
Pipette tips	Need to fit with pipettes
EP tubes	1.5 mL,10 mL, for sample dilution
Reagent bottle	For diluted wash solution, usually 500 mL, 1 L is recommended
Deionized or distilled water	For dilution of the solution, for example, dilute the 20×Washing Buffer to 1×Washing Buffer
Timer	For time control
Incubator	For plate incubation reaction, if the room temperature does not reach 20-25℃, it is recommended to put a 25℃ incubator.
Orbital microtiter plate shaker	For shaking the plate in immunological steps.
Microtiter plate reader spectrophotometer	Single or dual wavelength microplate reader with 450nm and 630nm filter (If your plate reader does not provide dual wavelength analysis, you can read at just the 450nm wavelength.)

Shipping and Storage

1. The product is shipped at room temperature.
2. Store the unopened kit at 2-8 °C.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

Precautions

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Wear appropriate personal protective apparel, please be careful and avoid to contact the reagent with your skin, eyes and clothing. In case of accidental skin exposure, flush with water immediately. Consult a physician if required.
3. Do not use the kit and the all reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. Activity of the conjugate is affected by nucleophiles such as azide, cyanide, and hydroxylamine.
6. If samples generate values higher than the highest standard, dilute the samples with the Dilution Buffer provided in kit and repeat the assay.
7. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.

Preparation before experiment

1. Experimental environment preparation

In order to ensure the accuracy of the experiment, the experimental environment requires that the operation process does not introduce additional Antibody or protein A. Prepare a clean test bench and tools.

2. Equipment and tools preparation

1) Prepare the necessary equipment, tools, reagents bottles and other utensils follow the table in “Materials & Equipment Required But Not Provided”.

2) Complete washing of the antibody coated plate to remove excess unreacted reagents is essential to good assay reproducibility and sensitivity. If you have an automatic washing machine for washing plates, this will save washing time and speed up the experiment. If you don't have an automatic washing machine, you can wash plate by manual wash procedure with a multichannel pipettor. A thorough washing procedure generally provides lower backgrounds, higher specific absorbance, and better precision.

Reagent Preparation

1. Take out the kit, equilibrate all reagents and samples to room temperature (20°C-25°C) before use, check that each buffer and standard solution are clear and transparent, make sure these solution are evenly mixed.

   Note: RES029-C07 component does not clarify and RES029-C06 component is easy to crystallize at lower temperatures (2-8°C), so be sure to balance the two components at room temperature until the liquid is clarified.
2. Reconstitute the provided lyophilized materials to stock solutions with sterile deionized water as recommended in the following table, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortex. The reconstituted stock solutions should be stored at -70°C. Avoid freeze-thaw cycles.

   Note: Streptavidin-HRP stock solution should be protected from light.

Catalog	Component	Amount	Stock Solution Conc.	Reconstitution Buffer and Vol.
RES029-C05	Streptavidin-HRP	10μg	100 μg/mL	100 μL water

Procedure for assay

1. **Prepare 1×Washing Buffer** by diluting the 20×Washing Buffer with distilled or deionized water.

   Calculate the required 1×Washing Buffer volume, for example, when 1 L of 1×Washing Buffer is required, prepare the 1×Washing Buffer by diluting 50 mL of the supplied 20×Washing Buffer with 950 mL of distilled or deionized water. It is recommended to prepare the1×Washing Buffer according to the experimental dosage, and use it up on the same day.

2. **Prepare 1×Sample Dilution Buffer** by diluting the 10×Sample Dilution Buffer with distilled or deionized water.

   Calculate the required 1×Sample Dilution Buffer volume, for example, when 100 mL of 1×Sample Dilution Buffer is required, prepare the 1×Sample Dilution Buffer by diluting 10 mL of the supplied 10×Sample Dilution Buffer with 90 mL of distilled or deionized water. It is recommended to prepare the1×Sample Dilution Buffer according to the experimental dosage, and use it up on the same day.

3. **Prepare the protein A standards**

   The resDetect™ Universal Protein A Quick ELISA Kit (Boiling-free) is compatible with recombinant Protein A samples and unnatural Protein A constructs such as alkaline-resistant Protein A variants or PrismA in neutralized buffers. The kit contains several types of protein A standards, please choose the appropriate standard to establish standard curve according to the affinity resin you are using for sample purification.

   For Mabselect SuRe™ or some other similar alkaline-resistant resins, such as UniMab® 50 Protein A (NanoMicro), Alkali-tolerant Recombinant Protein A Standard (RES029-C02A) is recommended directly for standard curve establishment.

   For MaXtar® ARPA ligand (Bio-Link Co.) resin, MaXtar® ARPA ligand Protein A Standard (RES029-C02B) is recommended directly for standard curve establishment.

   For MabSelect PrismA™ resin, Recombinant PrismA Standard (RES029-C02C) is recommended directly for standard curve establishment.

   If you use protein A resin coupled with other protein A variants that have not been included in this kit as standard, it is recommended to obtain the specific protein A variant standard solution from the purification resin supplier and store it under the recommended conditions, then follow the kit instruction to dilute it to the concentration range of the standard curve during use for quantitation. If the specific protein A variant is not available from resin supplier, just choose the protein A standard in the kit that is closest to the specific variant to establish the standard curve.

   Each well requires 25 μL of standard according to the method. Serially dilute the 1 μg/mL of protein A standard stock solution with 1×Sample Dilution Buffer to prepare your standards.

   Note: *Diluted standards should be used within 30 minutes of preparation.*

   All diluted standards must be denatured with Denaturation Buffer (RES029-C07). See "Prepare the samples" section for details.

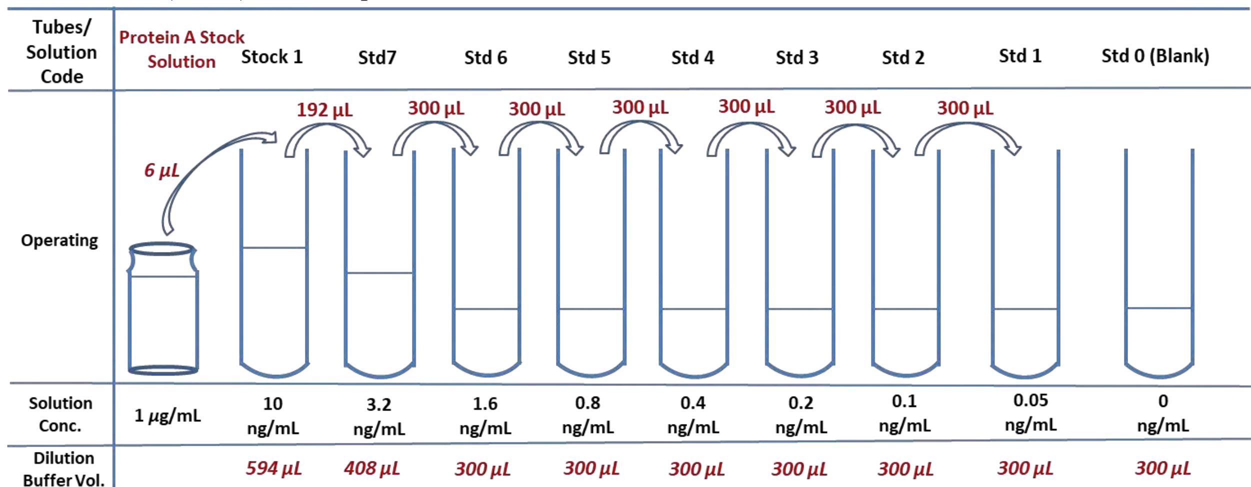
   In order to counteract any standard sticking, we recommend changing tips between each dilution.

   The recommended protein A standard dilution procedure is listed and illustrated below:

   1) Select appropriate protein A standards in the kit according to type of protein resins you used.
   2) Bring the protein A standard stock solution to room temperature, the original concentration is 1 μg/mL.
   3) Dilute the 1 μg/mL of standard stock solution 100-fold with 1×Sample Dilution Buffer to 10 ng/mL (Stock1).
   4) Dilute the 10 ng/mL of standard stock solution1 (Stock1) 3.125-fold with 1×Sample Dilution Buffer to 3.2 ng/mL, this yields the highest standard 7 **(Std 7: 3.2 ng/mL)** for the top of the Recombinant PrismA Standard (RES029-C02C) curve.

5) Dilute the 3.2 ng/mL of the standard 7 (Std 7) 2-fold with 1×Sample Dilution Buffer to 1.6 ng/mL, this yields the highest standard 6 (Std 6: 1.6 ng/mL) for the top of the Alkali-tolerant Recombinant Protein A Standard (RES029-C02A), MaXtar® ARPA ligand Protein A Standard (RES029-C02B) and Recombinant Protein A Standard (RES029-C03) curve.

6) Use the 1.6 ng/mL of the standard (Std 6) to prepare the standard curve using 2-fold serial dilutions as follows. After each step of dilution, the remaining volume of the standards should not be less than 100μL. (take 600μL of each concentration of standards as example):
- Dispense 300μL of 1×Sample Dilution Buffer into each vial from Std 5 to Std 1;
- Add 300 μL of protein A Std 6 to 300μL of 1×Sample Dilution Buffer, mix gently and repeat the serial dilution to make protein A standard solutions: Std 5, Std 4, Std 3, Std 2, Std 1, this will create 7 standards for the analyte;
- Std 0 (Blank) is 1×Sample Dilution Buffer alone.



Standard	Diluent Ratio	Serial Dilutions	Concentration
Stock solution (Stock1)	100	6 μL 1μg/mL Protein A Stock Solution + 594 μL 1×Sample Dilution Buffer	10 ng/mL
Standard 7	3.125	192 μL Stock1 + 408 μL 1×Sample Dilution Buffer	3.2 ng/mL
Standard 6	2	300 μL Standard 7 + 300 μL 1×Sample Dilution Buffer	1.6 ng/mL
Standard 5	2	300 μL Standard 6 + 300 μL 1×Sample Dilution Buffer	0.8 ng/mL
Standard 4	2	300 μL Standard 5 + 300 μL 1×Sample Dilution Buffer	0.4 ng/mL
Standard 3	2	300 μL Standard 4 + 300 μL 1×Sample Dilution Buffer	0.2 ng/mL
Standard 2	2	300 μL Standard 3 + 300 μL 1×Sample Dilution Buffer	0.1 ng/mL
Standard 1	2	300 μL Standard 2 + 300 μL 1×Sample Dilution Buffer	0.05 ng/mL
Standard 0	-	300μL 1×Sample Dilution Buffer	0 ng/mL

4. **Prepare the samples**

Aliquot a minimum of 100 μL of each sample and standard into a microcentrifuge tube. Add 50 μL of Denaturation Buffer (RES029-C07) to each tube. Mix by pipetting up and down ~15 times or mix gently on a vortex mixer. Use fresh tips for each addition. Stand for 5-10min.

Note: The recovery rate of each testing sample shall be determined:

1) *All samples with a concentration of protein A above the highest standard must be diluted, when the total amount of added protein A and endogenous Protein A from the sample itself above the highest standard, the samples also need to be diluted to a reasonable concentration, or your sample contains interfering ingredients, it also needs to be diluted to reduce interference.*
2) *When samples need to be diluted, dilute the samples with the 1×Sample Dilution Buffer to yield acceptable background and not impurities with Protein A, sample dilution should be performed prior to the sample denaturation step for best results.*
3) *The diluted samples should also give acceptable recovery when spiked with known quantities of Protein A, when the recovery rate is in the range of 80% to 120%, it indicates that the detection value of the diluted sample is reliable.*
4) *This experiment can be performed by add a certain concentration of Protein A beyond the linear range to the samples, then dilute the sample to a reasonable range, this experiment also can be performed by spiking a standard provided with this kit with concentration in the linear range into the testing samples, for example, adding 1 part of the 0.8 ng/mL, 0.4 ng/mL or 0.2 ng/mL standard to 1 part of a 2mg/mL of test sample. This yields an added spike of 0.4 ng/mL, 0.2 ng/mL and 0.1 ng/mL, any endogenous Protein A from the sample itself determined prior to spiking and corrected for by the 50% dilution of that sample should be subtracted from the value determined for the spiked sample, then calculated the concentration of protein A to give the recovery rate. If the protein A content of the sample itself exceeds the highest standard, dilute the sample to a linear concentration and then add standards for recovery:*

Sample Recovery ID	Diluent Ratio	Sample and Standard Volume	Final Concentration of Sample	Final Concentration of Protein A
Sample 1-R1	2	150 μL Standard 5 + 150 μL test sample	1 mg/mL	0.4 ng/mL
Sample 1-R2	2	150 μL Standard 4 + 150 μL test sample	1 mg/mL	0.2 ng/mL
Sample 1-R3	2	150 μL Standard 3 + 150 μL test sample	1 mg/mL	0.1 ng/mL
Sample 2-R1	4	150 μL Standard 5 + 150 μL Sample 1	0.5 mg/mL	0.4 ng/mL
Sample 2-R2	4	150 μL Standard 4 + 150 μL Sample 1	0.5 mg/mL	0.2 ng/mL
Sample 2-R3	4	150 μL Standard 3 + 150 μL Sample 1	0.5 mg/mL	0.1 ng/mL

5. **Prepare the Biotin-Anti-Protein A Antibody working solution**

Each well requires 100 μL of Biotin-Anti-Protein A Antibody working solution.

Calculate the required total volume of Biotin-Anti-Protein A Antibody working solution according to the wells number in the experiment. Dilute the Biotin-Anti-Protein A Antibody stock solution 10-fold with Antibody Dilution Buffer (RES029-C09). For example, When the number of experimental wells is 96, 9.6 mL of Biotin-Anti-Protein A Antibody working solution is required, we can prepare 11 mL of working solution to ensure a margin, add 1100 μL Biotin-Anti-Protein A Antibody into 9900 μL Antibody Dilution Buffer.

Please refer to the following methods to prepare the solution:

Tests	Working solution	Biotin-Anti-Protein A Antibody stock solution	Antibody Dilution Buffer
96 Tests	11000 μL	1100 μL	9900 μL

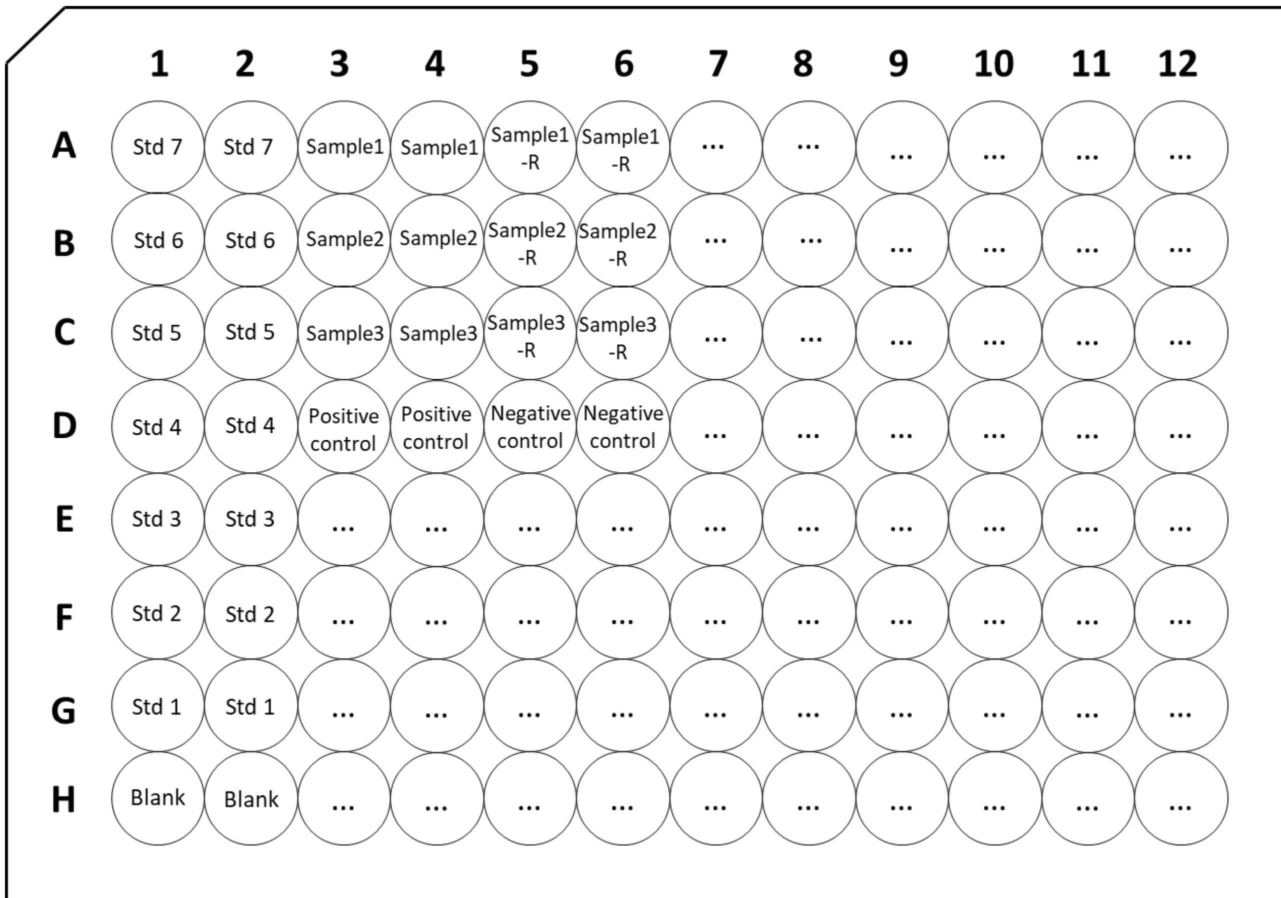
6. **Add Biotin-Anti-Protein A Antibody working solution and Samples**

Take out the Pre-coated Anti-Protein A Antibody Microplate, bring the plate to room temperature, add the above prepared Biotin-Anti-Protein A Antibody working solution and samples, protein A standards to the plate wells as required:

Add 100μL Biotin-Anti-Protein A Antibody working solution to each well, then add 25μL samples or standards to each well, seal the plate with microplate sealing film and incubate at room temperature (20°C-25°C) for 1 hour on orbital shaker at 400-600 rpm.

It is recommended that each concentration of standards and your samples be reperforated. If you need to add your own positive references and negative references, the number of wells for the positive references should be no less than 1, and the number of wells for the negative references should be no less than 2:

Note: *The standards, controls and all test samples should be treated in exactly the same way and measured in the same plate.*



7. **Washing**

Thorough washing is essential to proper performance of this assay. Automated plate washing systems or manual wash procedure be selected according to your own experimental conditions.

Remove the remaining solution of the wells, wash the wells by add 300 µL of 1×Washing Buffer to each well, gently tap the plate for 1 min, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against lint free paper towels to remove any remaining wash buffer. Please note that the complete removal of the washing buffer is essential.

Repeat the wash step above for 3 times.

8. **Add Streptavidin-HRP Solution**

Each well requires 100 µL of Streptavidin-HRP working solution. Calculate the required total volume of Streptavidin-HRP working solution according to the wells number in the experiment. Dilute the Streptavidin-HRP stock solution 500-fold with Streptavidin-HRP Dilution Buffer (RES029-C10). For example, When the number of experimental wells is 96, 9.6 mL of Streptavidin-HRP working solution is required, we can prepare 11 mL of working solution to ensure a margin, add 22 µL Streptavidin-HRP stock solution into 10978 µL Streptavidin-HRP Dilution Buffer.

Please refer to the following methods to prepare the solution. Add 100 µL Streptavidin-HRP working solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20°C-25°C) for 30 min on orbital shaker at 400-600 rpm, avoid light.

Tests	Working solution	Streptavidin-HRP stock solution stock solution	Streptavidin-HRP Dilution Buffer
96 Tests	11000 μL	22 μL	10978 μL

9. **Washing**
   Repeat step 7.
10. **Substrate Reaction**
    Add 100 μL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20°C-25°C) for 20 min, avoid light. Do not shake.
11. **Termination**
    Add 50 μL Stop Solution to each well and tap the plate gently to allow thorough mixing.
    Note: *the color in the wells should change from blue to yellow.*
12. **Data Recording**
    Read the absorbance at 450nm and 630nm using UV/Vis microplate spectrophotometer.
    Note: *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

Calculation of Results

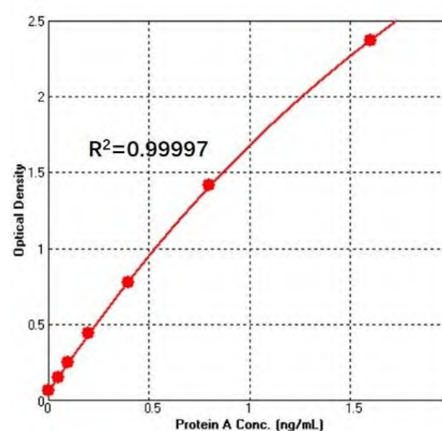
1. Calculate the mean absorbance for each standard, control and sample and subtract blank control standard optical density (OD.).
2. The standard curve is plotted with the standard concentration as x-axis (abscissa) and the calibrated absorbance value as y-axis (ordinate). Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: the correlation coefficient R^2 should be ≥ 0.9900.
4. Detection range: 0.05 ng/mL-1.6 ng/mL (Protein A), 0.05 ng/mL-3.2 ng/mL (PrismA). If the OD value of the sample to be tested is higher than the highest standard (1.6 ng/mL for Protein A or 3.2ng/mL for PrismA), the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.05 ng/mL, the sample residual should be reported < 0.05ng/mL.

Typical Data

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only.

Standard curve of Recombinant Protein A:

Standard Num.	Concentration	O.D.
Standard 6	1.6 ng/mL	2.372
Standard 5	0.8 ng/mL	1.414
Standard 4	0.4 ng/mL	0.781
Standard 3	0.2 ng/mL	0.442
Standard 2	0.1 ng/mL	0.253
Standard 1	0.05 ng/mL	0.155
Standard 0	0 ng/mL	0.065



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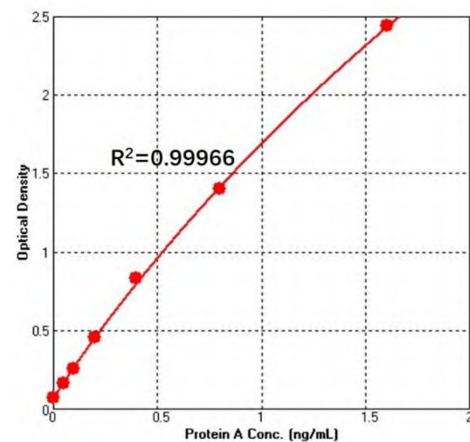
http://www.acrobiosystems.com

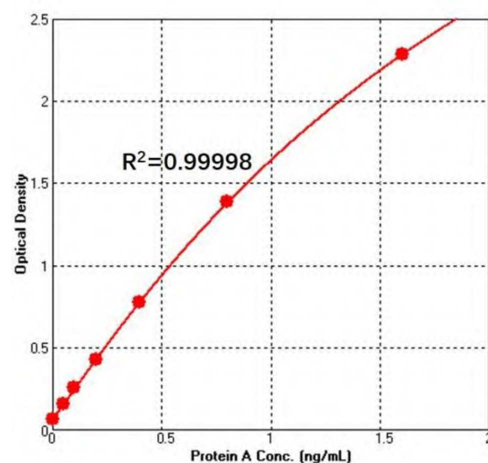
Standard curve of Alkali-tolerant Recombinant Protein A:

Standard Num.	Concentration	O.D.
Standard 6	1.6 ng/mL	2.437
Standard 5	0.8 ng/mL	1.401
Standard 4	0.4 ng/mL	0.832
Standard 3	0.2 ng/mL	0.455
Standard 2	0.1 ng/mL	0.256
Standard 1	0.05 ng/mL	0.170
Standard 0	0 ng/mL	0.078



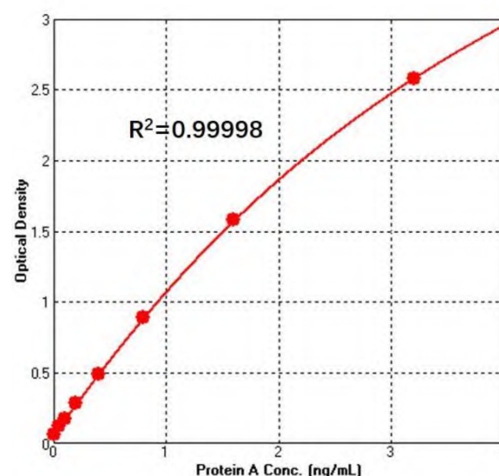
Standard curve of MaXtar® ARPA ligand Protein A (Bio-Link Co.):

Standard Num.	Concentration	O.D.
Standard 6	1.6 ng/mL	2.285
Standard 5	0.8 ng/mL	1.390
Standard 4	0.4 ng/mL	0.778
Standard 3	0.2 ng/mL	0.433
Standard 2	0.1 ng/mL	0.256
Standard 1	0.05 ng/mL	0.161
Standard 0	0 ng/mL	0.069

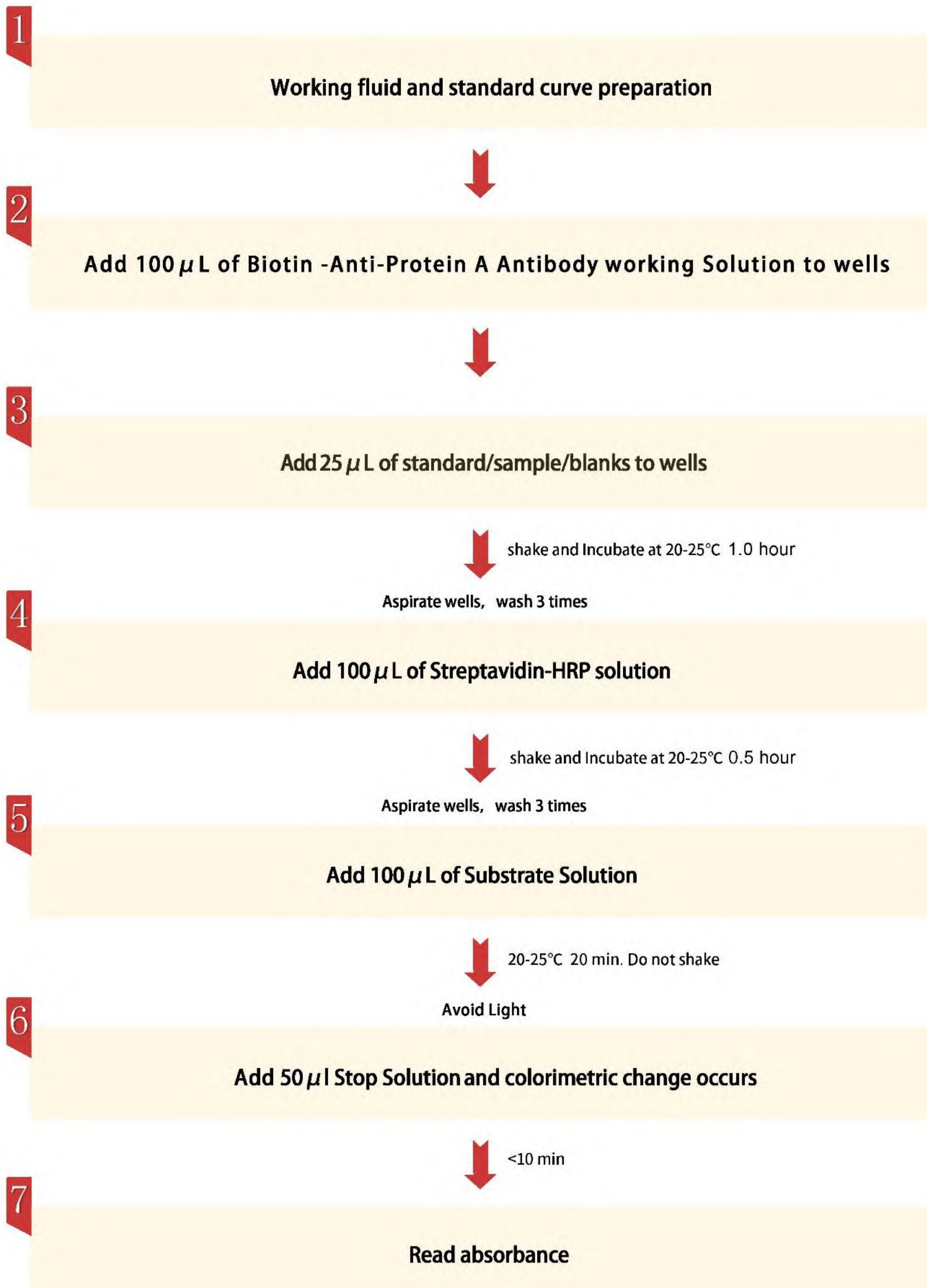


Standard curve of Recombinant PrismA:

Standard Num.	Concentration	O.D.
Standard 7	3.2 ng/mL	2.582
Standard 6	1.6 ng/mL	1.579
Standard 5	0.8 ng/mL	0.887
Standard 4	0.4 ng/mL	0.486
Standard 3	0.2 ng/mL	0.282
Standard 2	0.1 ng/mL	0.176
Standard 1	0.05 ng/mL	0.120
Standard 0	0 ng/mL	0.067



Quickguide



Performance Characteristics

Sensitivity

Standard	Assay range (ng/mL)	Limit of quantification (LoQ*)
Recombinant Protein A	0.05-1.6 ng/mL	0.05 ng/mL
Alkali-Tolerant Recombinant Protein A	0.05-1.6 ng/mL	0.05 ng/mL
MaXtar® ARPA ligand Protein A (Bio-Link Co.)	0.05-1.6 ng/mL	0.05 ng/mL
Recombinant PrismA	0.05-3.2 ng/mL	0.05 ng/mL

Precision and Accuracy（Intra-Assay）

Standard	Sample Conc.(ng/mL)	Replicate Times	Mean (ng/mL)	SD	CV (%)	Recovery (%)
Recombinant Protein A	1.6	10	1.65	0.167	10.10%	103%
	1.2	10	1.35	0.122	9.00%	113%
	0.3	10	0.3	0.015	5.10%	100%
	0.12	10	0.13	0.01	7.70%	105%
	0.05	10	0.05	0.007	14.90%	99%
Alkali-Tolerant Recombinant Protein A	1.6	10	1.6	0.075	4.70%	100%
	1.2	10	1.2	0.056	4.60%	100%
	0.3	10	0.29	0.013	4.60%	98%
	0.12	10	0.11	0.005	4.00%	95%
	0.05	10	0.04	0.004	9.50%	87%
MaXtar® ARPA ligand Protein A Standard (Bio-Link Co.)	1.6	10	1.52	0.054	3.60%	95%
	1.2	10	1.16	0.049	4.20%	97%
	0.3	10	0.27	0.014	5.20%	90%
	0.12	10	0.1	0.009	8.90%	83%
	0.05	10	0.04	0.003	7.30%	80%
Recombinant PrismA	3.2	10	0.030	0.055	2.99%	93%
	2.4	10	0.021	0.032	2.14%	95%
	0.5	10	0.023	0.009	2.30%	93%
	0.1	10	0.032	0.005	3.23%	93%
	0.05	10	0.119	0.014	11.92%	115%

Note: *The example data is for reference only.*

Precision and Accuracy（Inter-Assay）

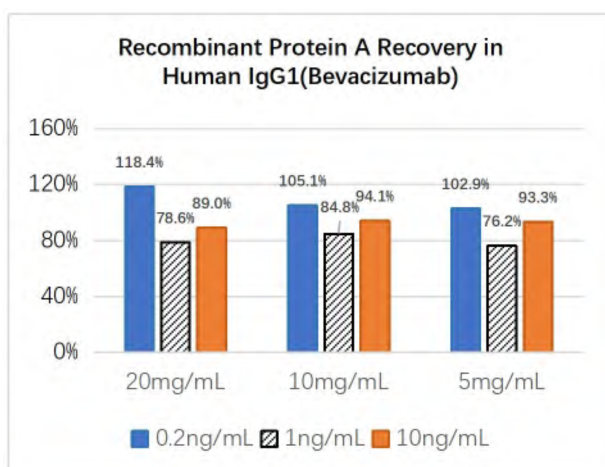
Standard	Sample Conc.(ng/mL)	Replicate Times	Mean (ng/mL)	SD	CV (%)	Recovery (%)
Recombinant Protein A	1.6	10	1.66	0.079	4.70%	104%
	1.2	10	1.23	0.1	8.10%	103%
	0.3	10	0.32	0.018	5.50%	106%
	0.12	10	0.13	0.013	10.20%	105%
	0.05	10	0.05	0.006	12.40%	96%
Alkali-Tolerant Recombinant Protein A	1.6	10	1.69	0.067	3.90%	106%
	1.2	10	1.35	0.054	4.00%	113%
	0.3	10	0.32	0.02	6.30%	107%
	0.12	10	0.13	0.011	8.40%	107%
	0.05	10	0.05	0.009	16.90%	104%
MaXtar® ARPA ligand Protein A Standard (Bio-Link Co.)	1.6	10	1.65	0.069	4.20%	103%
	1.2	10	1.23	0.04	3.20%	103%
	0.3	10	0.31	0.018	5.80%	103%
	0.12	10	0.12	0.011	9.30%	100%
	0.05	10	0.05	0.004	7.80%	91%
Recombinant PrismA	3.2	10	3.11	0.091	2.93%	97%
	2.4	10	2.47	0.113	4.59%	103%
	0.5	10	0.47	0.047	10.00%	94%
	0.1	10	0.11	0.015	13.57%	110%
	0.05	10	0.05	0.008	16.10%	93%

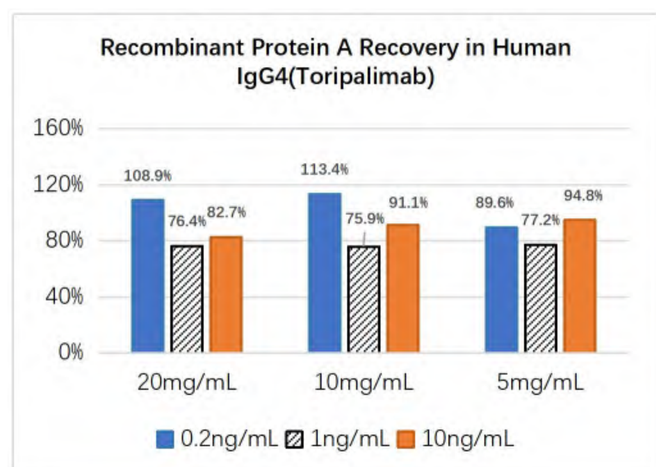
Note: *The example data is for reference only.*

Recovery

Add different concentrations of Protein A (0.2ng/mL、1ng/mL、10ng/mL) to different concentrations of Human IgG1 (Bevacizumab) (20mg/mL、10mg/mL、5mg/mL) or Human IgG4 (Toripalimab) (20mg/mL、10mg/mL、5mg/mL), then dilute the antibodies to a reasonable range, then test and calculated the concentration of protein A to give the recovery rate.

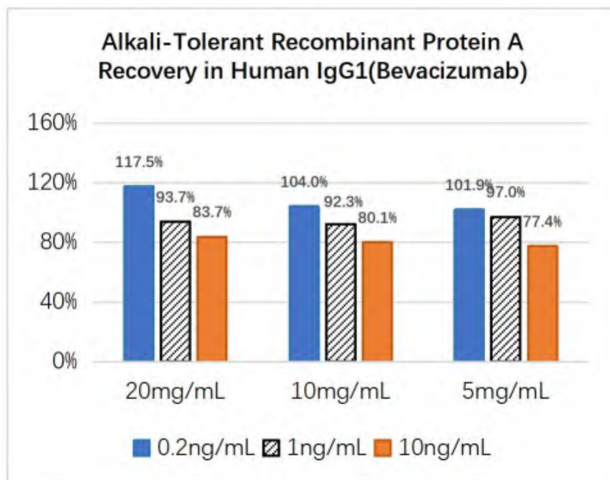
Add Recombinant Protein A to Human IgG1 (Bevacizumab) or Human IgG4 (Toripalimab):

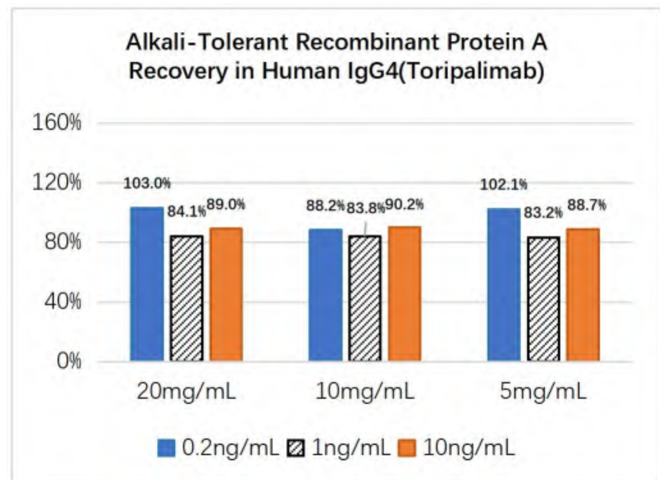




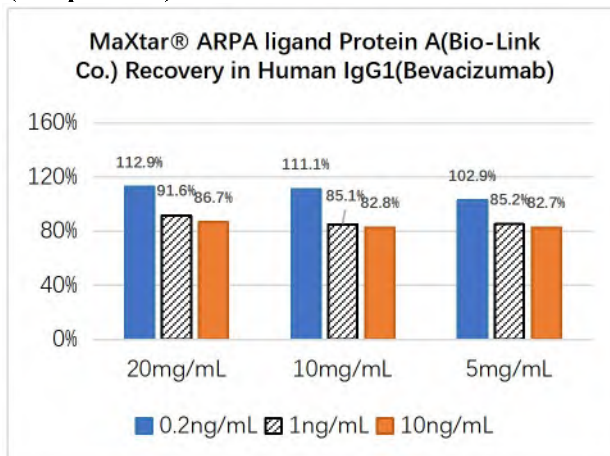
If you have any questions, please contact our technical support team at: TechSupport@acrobiosystems.com
http://www.acrobiosystems.com

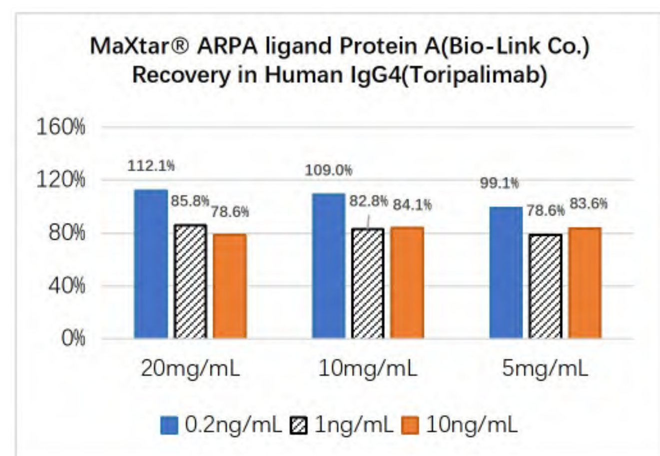
Add Alkali-Tolerant Recombinant Protein A to Human IgG1 (Bevacizumab) or Human IgG4 (Toripalimab):



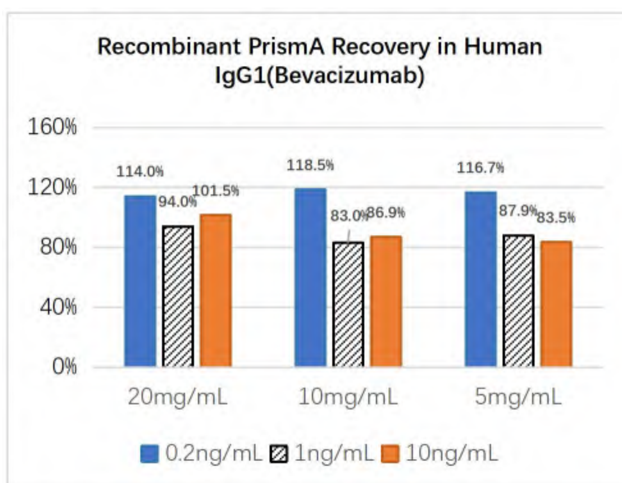


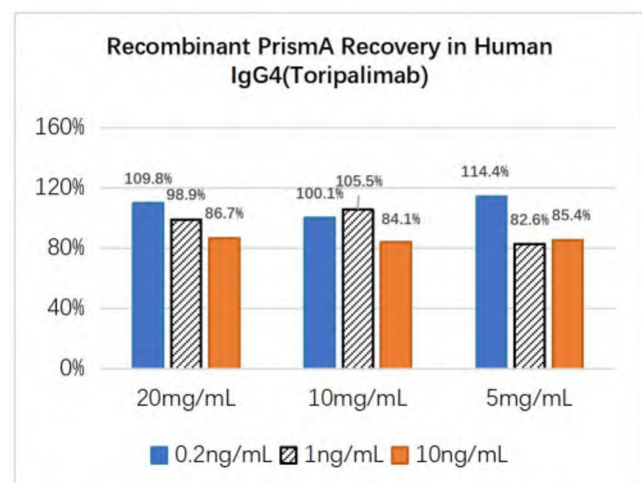
Add MaXtar® ARPA ligand Protein A (Bio-Link Co.) to Human IgG1 (Bevacizumab) or Human IgG4 (Toripalimab):





Add Recombinant PrismA to Human IgG1 (Bevacizumab) or Human IgG4 (Toripalimab):





Interfering Substances

We have conducted interference effect test about frequently-used buffers, they have excellent buffer compatibility. For specific buffers, it is recommended that you verify recovery to determine the minimum dilution ratio.

	Recombinant Protein A		Alkali-Tolerant Recombinant Protein A		MaXtar® ARPA ligand Protein A (Bio-Link Co.)		Recombinant PrismA	
Matrix	**Recovery**	**Dilution Factor**	**Recovery**	**Dilution Factor**	**Recovery**	**Dilution Factor**	**Recovery**	**Dilution Factor**
20mM L-histidine with 0.1% (w/v) PF68, pH6.0	116%	1	105%	1	116%	1	110%	1
20mM L-histidine with 0.4% (w/v) Tween-80, pH6.0	105%	1	92%	1	105%	1	102%	1
1×PBS, pH7.3	95%	2	96%	1	95%	2	103%	2
1*PBS, pH7.3 with 11% Trehalose	93%	2	96%	2	93%	2	88%	2
20mM L-histidine, pH6.0	100%	2	97%	2	100%	2	97%	2
50mM Tris,100mM Glycine, pH7.5	96%	2	98%	2	96%	2	96%	2
100mM Tris,20mM Sodium citrate, pH7.5	91%	2	105%	1	91%	2	86%	2
20mM L-histidine 10% trehalose,pH6.0	90%	2	108%	2	90%	2	94%	2
50 mM Na Acetate, pH 3.5	109%	2	88%	1	109%	2	91%	2
25 mM Phosphate, pH 7.5	117%	2	105%	2	117%	2	88%	2
100 mM Glycine, pH 3.5	91%	1	97%	2	91%	1	88%	2
100 mM Triscitrate, 7.5	97%	2	107%	2	97%	2	90%	1
100 mM Trisacetate, 7.5	113%	2	109%	2	113%	2	92%	1

Specificity

Host cell protein (HCP 500 ng/mL) and host cell DNA (HCD 0.5 ng/mL) of HEK293, E.coli or CHO systems were added to human IgG1 (Bevacizumab, 1mg/mL) and human IgG4 (Toripalimab, 1mg/mL), respectively, which were higher than the usual quality standard limit. Then high, medium, and low concentrations of Protein A were added, respectively, and the ratio of Protein A recovery in the Protein A added samples without HCP and HCD was added as the specificity verification index. The calculation formula was as follows: (S3-S1) / (S2-S1) × 100%, the experimental design is as follows:

Standard	**ID**	**Sample ID**	**Antibody Conc.(mg/mL)**	**Protein A Conc.(ng/mL)**	**HCP Conc. (ng/mL)**	**HCD Conc. (ng/mL)**
	S1	S1	1	0	0	0
Recombinant Protein A, Alkali-Tolerant Recombinant Protein A or MaXtar® ARPA ligand Protein A (Bio-Link Co.)	S2	S2-1	1	1.6	0	0
		S2-2	1	0.3	0	0
		S2-3	1	0.05	0	0
	S3	S3-1	1	1.6	500	0.5
		S3-2	1	0.3	500	0.5
		S3-3	1	0.05	500	0.5

Recombinant PrismA	S2	S2-1	1	3.2	0	0
		S2-2	1	0.5	0	0
		S2-3	1	0.05	0	0
	S3	S3-1	1	3.2	500	0.5
		S3-2	1	0.5	500	0.5
		S3-3	1	0.05	500	0.5

The results are as follows:

Sample	**Antibody Conc. (mg/mL)**	**Protein A Conc. (ng/mL)**	**HCP Conc. (ng/mL)**	**HCD Conc. (ng/mL)**	**Bevacizumab**			**Toripalimab**		
					HEK293	**E.coli**	**CHO**	**HEK293**	**E.coli**	**CHO**
Specificity of (Recombinant Protein A) Recovery	1	1.6	500	0.5	97.2%	79.8%	93.7%	87.6%	103.2%	102.1%
	1	0.3	500	0.5	92.3%	97.3%	97.1%	83.5%	106.5%	106.7%
	1	0.05	500	0.5	78.5%	97.0%	115.7%	82.9%	109.2%	113.7%
Specificity of (Alkali-Tolerant Recombinant Protein A) Recovery	1	1.6	500	0.5	101.3%	109.8%	96.1%	103.4%	90.8%	84.8%
	1	0.3	500	0.5	89.7%	105.5%	99.4%	101.2%	87.2%	101.5%
	1	0.05	500	0.5	82.5%	112.7%	92.5%	91.0%	85.5%	101.1%
Specificity of (MaXtar® ARPA ligand Protein A (Bio-Link Co.) Recovery	1	1.6	500	0.5	86.0%	98.4%	106.7%	88.8%	106.2%	96.4%
	1	0.3	500	0.5	99.8%	95.1%	105.5%	98.4%	97.7%	98.4%
	1	0.05	500	0.5	110.9%	86.6%	114.4%	103.6%	119.9%	77.6%
Specificity of (Recombinant PrismA) Recovery	1	3.2	500	0.5	88.1%	102.6%	101.1%	85.0%	106.0%	107.5%
	1	0.5	500	0.5	97.6%	100.5%	100.3%	97.5%	100.2%	105.5%
	1	0.05	500	0.5	82.4%	95.7%	118.0%	74.4%	104.1%	109.8%

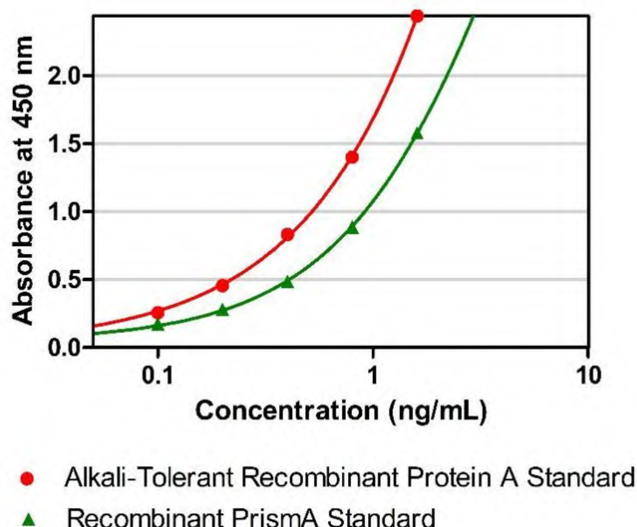
Troubleshooting

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain Protein A levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

Frequently asked questions (FAQs)

1. Which Protein A ligands are RES-A029 suitable for?
   The RES-A029 is suitable for detection of natural or structurally conserved recombinant forms of Protein A and alkaline-resistant Protein A variants, such as MabSelect SuRe™, MaXtar® ARPA ligand (Bio-Link Co.), UniMab® 50 Protein A (NanoMicro), MabSelect PrismA™ and other ligands. If you use protein A resin coupled with other protein A variants that have not been included in this kit as standard, it is recommended to obtain the specific protein A variant standard solution from the purification resin supplier and store it under the recommended conditions, then follow the kit instruction to dilute it to the concentration range of the standard curve during use for quantitation. If the specific protein A variant is not available from resin supplier, just choose the protein A standard in the kit that is closest to the specific variant to establish the standard curve.
2. Why is it necessary to use the protein A standard that matches the protein A resin for protein A residue detection?
   According to the requirements of the USP 1103 regarding the ELISA assay method "Quantitative assays determine the quantity of the analyte based on the interpolation of a standard calibration curve with known analyte concentration, run simultaneously in the same assay. This standard should be an appropriate, preferably homologous, reference or calibration material that is representative of the analyte(s) of interest." Regulatory authorities recommend using matching standard products.
   As shown in the following figure, the difference in absorbance values is obvious from alkaline-resistant Protein A and PrismA. If Alkali-Tolerant Recombinant Protein A curve is used to detect samples that containing PrismA, the residual value will be low (false negative) and the accuracy of detection will be greatly compromised. Therefore, in order to obtain more accurate results, it is recommended to use a standard that

matches the resin to calibrate the determination of the sample.



3. What is the quantitative method for the standard substance in the RES-A029 assay kit?
   The standard raw materials of the kit have a high concentration, which are quantified by UV. Then the standard raw materials are accurately diluted to 1μg/mL.
4. How to ensure the accuracy of spiked sample detection in Protein A residue detection experiments?
   For samples with Protein A residues, as the residual amount cannot be predicted in advance, it is recommended to start diluting the sample from a low dilution ratio, and add a standard substance at a certain intermediate concentration point for spiking detection at each dilution ratio to calculate the spiking recovery rate. In principle, the amount of added standards should be as close or equal to the content of the test substance as possible, and should not exceed three times the content of the test substance. The measured value after adding standards should not exceed 90% of the upper limit of the method.
   As shown in the following table, the residual value of Protein A in the sample diluted twice is 1.559ng/mL, which is close to the upper limit of quantification. When the spiked amount is 0.2ng/mL, the spiked amount is much lower than the residual amount, so the recovery rate is not accurate. By increasing the dilution ratio, the detection value gradually decreases and approaches the spiked amount after dilution, so as to obtain a perfect spiked recovery rate.

Sample ID	Dilute factor	Samples			Standard added Samples			Recovery
		OD450	Protein A back Calculated Conc. (ng/ml)	Protein A final Conc. (ng/ml)	Added Protein A (ng/ml)	OD450	Protein A back Calculated Conc. (ng/ml)	
MAb1	2	1.79	1.559	3.118	0.20	1.879	1.664	52.5%
	4	0.852	0.614	2.456	0.20	1.106	0.844	115.0%
	8	0.489	0.312	2.496	0.20	0.779	0.551	119.4%
	16	0.322	0.182	2.918	0.20	0.578	0.383	100.5%

5. Is it necessary to revalidate the methods for the different samples purified from the same resin chromatography column?
   Yes, it is. Different samples may have different interfering factors, so every sample should be revalidated the methods.