resDetect™ Universal Protein A Quick ELISA Kit

Cat. No. RES-A024 **Size: 96 tests**

Intended Use

The Universal Protein A Quick ELISA kit 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 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 China National Standard (NIFDC code: 140619) with validated pharmacopoeia quantitation methodology
- Extensive validation: Validation Report (ICH compliant) available on request
- High sensitivity - Sensitivity < 20 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 10 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.

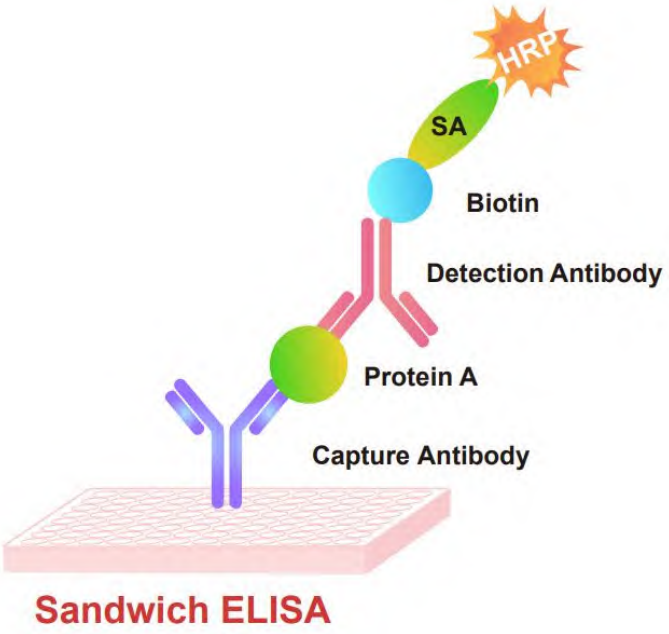
The Universal Protein A Quick ELISA Kit can detect protein A or unnatural protein A variants within 2 hours, it is high sensitive and easy to use. Whether in upstream small-scale trials or downstream large-scale of antibody production processes, this kit can help you to accurate analysis of samples, 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.), 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 Universal Protein A Quick ELISA kit 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 heated in a dry heating block or boiling water bath to dissociation of protein A and antibody, after a centrifugation step to pellet the antibody, add the standard samples and your samples supernatant to the plate, then add the Biotin-Anti-Protein A Antibody 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.



Reagents & Materials Provided

Catalog	Components	Size (96 tests)	Storage
RES024-C01	Pre-coated Anti-Protein A Antibody Microplate	1 plate	2-8℃
RES024-C02A	Alkali-tolerant Recombinant Protein A Standard (1 μg/mL)	100 μL	2-8℃
RES024-C02B	MaXtar® ARPA ligand Protein A Standard (Bio-Link Co.) (1μg/mL)	100 μL	2-8℃
RES024-C03	Recombinant Protein A Standard (1 μg/mL)	100 μL	2-8℃
RES024-C04	Biotin-Anti-Protein A Antibody	700 μL	2-8℃
RES024-C05	Streptavidin-HRP	300 μL	2-8℃, avoid light
RES024-C06	Dilution Buffer	100 mL	2-8℃
RES024-C07	20×Washing Buffer	30 mL	2-8℃
RES024-C08	Substrate Solution	12 mL	2-8℃, avoid light
RES024-C09	Stop Solution	6 mL	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.
Boiling water bath or dry heating block	For boiling samples
High Speedcentrifuge	Used to centrifuge heated samples (10000~12000xg)
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

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

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 the protein A standards**

The Universal Protein A ELISA kit is compatible with recombinant Protein A samples and unnatural Protein A constructs such as alkaline-resistant Protein A variants in neutralized buffers. The kit contains several types of protein A standards, please choose the approprite 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, Alkali-tolerant Recombinant Protein A Standard (RES024-C02A) is recommended directly for standard curve establishment.

For MaXtar® ARPA ligand (Bio-Link Co.) resin, MaXtar® ARPA ligand Protein A Standard (RES024-C02B) 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 50 µL of standard according to the method. Serially dilute the 1 µg/mL of protein A standard stock solution with the Dilution Buffer used to prepare your standards.

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

All diluted standards must be boiled. See "prepare the samples" section for details.

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

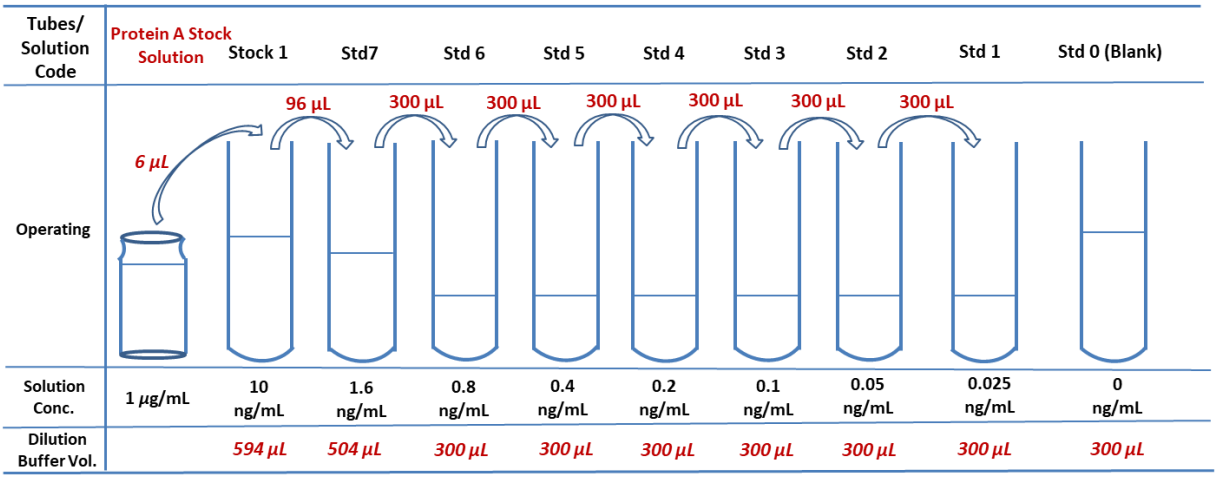
The recommended protein A standard dilution procedure is listed and illustrated below:

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 Dilution Buffer to 10 ng/mL (Stock1).
4) Dilute the 10 ng/mL of standard stock solution1 (Stock1) 6.25-fold with Dilution Buffer to 1.6 ng/mL, this

yields the high standard **(Std 7: 1.6 ng/mL)** for the top of the curve.

5) Use the high standard (Std 7) 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 0.3 mL. (take 600µL of each concentration of standards as example):

- Dispense 300µL of Dilution Buffer into each vial from Std 6 to Std 1;
- Add 300 µL of protein A Std 7 to 300µL of Dilution Buffer, mix gently and repeat the serial dilution to make protein A standard solutions: Std 6, Std 5, Std 4, Std 3, Std 2, Std 1, this will create 7 standards for the analyte;
- Std 0 (Blank) is Dilution Buffer alone.



Standard	Diluent Ratio	Serial Dilutions	Concentration
Stock solution (Stock1)	100	6 µL 1µg/mL Protein A Stock Solution + 594 µL Dilution Buffer	10 ng/mL
Standard 7	6.25	96 µL Stock1 + 504 µL Dilution Buffer	1.6 ng/mL
Standard 6	2	300 µL Standard 7 + 300 µL Dilution Buffer	0.8 ng/mL
Standard 5	2	300 µL Standard 6 + 300 µL Dilution Buffer	0.4 ng/mL
Standard 4	2	300 µL Standard 5 + 300 µL Dilution Buffer	0.2 ng/mL
Standard 3	2	300 µL Standard 4 + 300 µL Dilution Buffer	0.1 ng/mL
Standard 2	2	300 µL Standard 3 + 300 µL Dilution Buffer	0.05 ng/mL
Standard 1	2	300 µL Standard 2 + 300 µL Dilution Buffer	0.025 ng/mL
Standard 0	-	300µL Dilution Buffer	0 ng/mL

3. Prepare the samples

Aliquot a minimum of 0.3 mL of each sample and standard into a microcentrifuge tube with a hole in the lid (if there is no hole in the lid of the tube, a pinhole can be inserted into the lid of the centrifugal tube to release pressure during subsequent heating). This volume will allow for duplicates of each sample and standard to be measured in the assay. Include an additional tube with Dilution Buffer only (0 ng/mL).

Heat samples, the diluted standards, and blank buffer for 10-15 minutes in a boiling water bath or dry heating block. Cool the samples, standards and buffer for 8-10 minutes at room temperature. Centrifuge samples for 4 minutes at 10000 to 12,000 rpm/min at room temperature. You can transfer the supernatants to a new EP tube, mark it. You can also use supernatants from the cooled sample and standard tubes directly in the assay.

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

1) *All samples with a concentration of protein A above the highest standard (Std 7) must be diluted, when the total amount of added protein A and endogenous Protein A from the sample itself above the highest standard (Std 7), 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 Dilution Buffer provided in kit 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 concentrations 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 (Std 7), 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 6 + 150 µL test sample	1 mg/mL	0.4 ng/mL
Sample 1-R2	2	150 µL Standard 5 + 150 µL test sample	1 mg/mL	0.2 ng/mL
Sample 1-R3	2	150 µL Standard 4 + 150 µL test sample	1 mg/mL	0.1 ng/mL
Sample 2-R1	4	150 µL Standard 6 + 150 µL Sample 1	0.5 mg/mL	0.4 ng/mL
Sample 2-R2	4	150 µL Standard 5 + 150 µL Sample 1	0.5 mg/mL	0.2 ng/mL
Sample 2-R3	4	150 µL Standard 4 + 150 µL Sample 1	0.5 mg/mL	0.1 ng/mL

4. Prepare the Biotin-Anti-Protein A Antibody working solution

Each well requires 50 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 Dilution Buffer. For example, When the number of experimental wells is 96, 4.8 mL of Biotin-Anti-Protein A Antibody working solution is required, we can prepare 5.5 mL of working solution to ensure a margin, add 550 µL Biotin-Anti-Protein A Antibody into 4950 µL Dilution Buffer.

Please refer to the following methods to prepare the solution:

Tests	Working solution	Biotin-Anti-Protein A Antibody stock solution	Dilution Buffer
96 Tests	5500 µL	550 µL	4950 µL

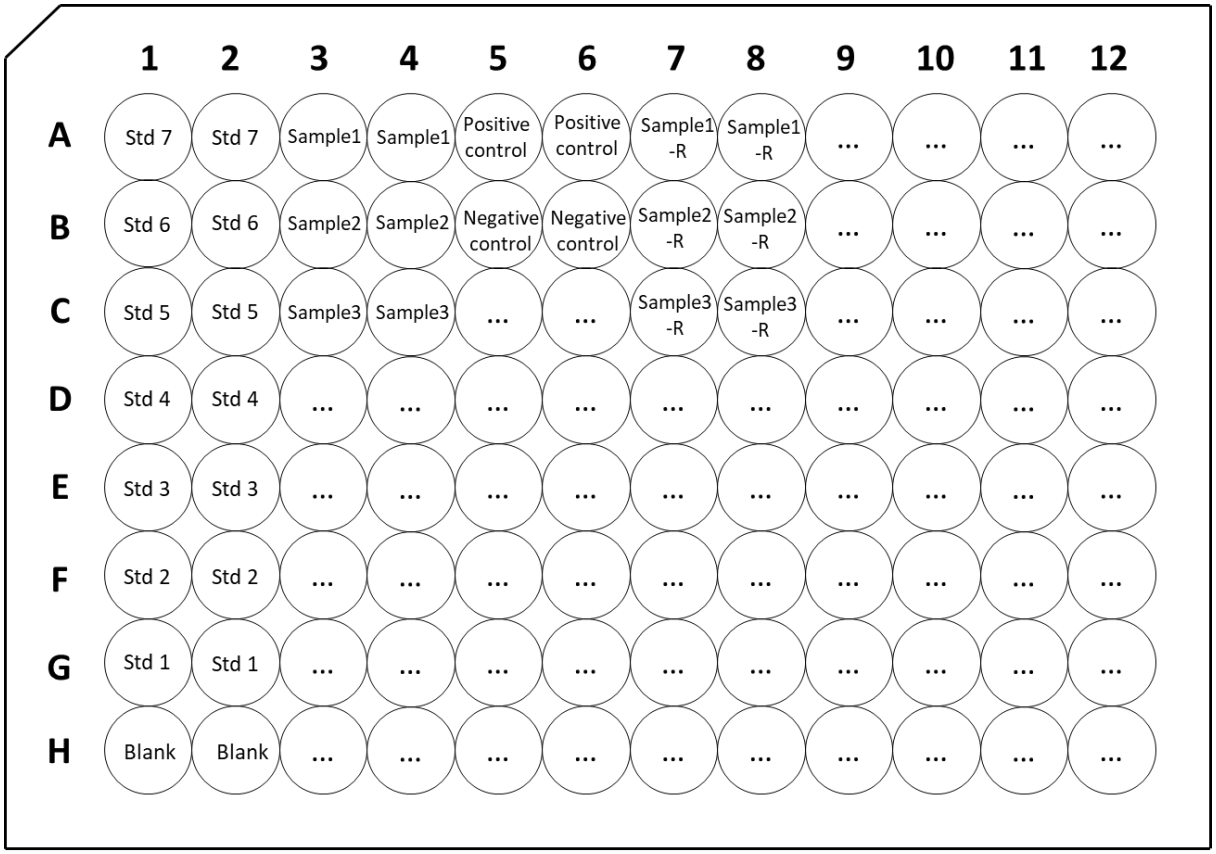
5. Add Samples and Biotin-Anti-Protein A Antibody working solution

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

Add 50µL samples or standards to each well, then add 50µL Biotin-Anti-Protein A Antibody working solution to each well, seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 1 hour.

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.*



6. 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.

7. 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 50-fold with Dilution Buffer. 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 0.22 mL Streptavidin-HRP stock solution into 10.78 mL Dilution Buffer.

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

Tests	Working solution	Streptavidin-HRP stock solution stock solution	Dilution Buffer
96 Tests	11 mL	0.22 mL	10.78 mL

8. **Washing**

   Repeat step 6.

9. **Substrate Reaction**

   Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (20℃-25℃) for 20 min, avoid light.

10. **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.*

11. **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

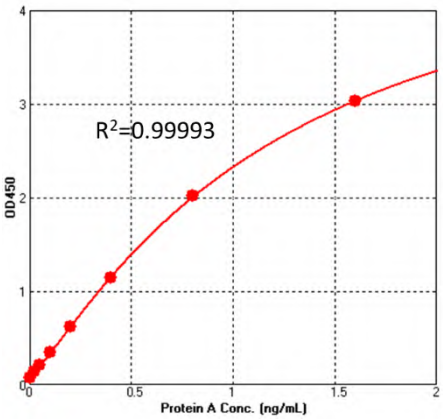
1. Calculate the mean absorbance for each standard, control and sample and subtract blank control standard optical density (O.D.).
2. The standard curve is plotted with the standard concentration as x-axis (abscissa) and the calibrated absorbance value as y-axis (ordinate). Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: the correlation coefficient R^2 should be $\geqslant$ 0.9900.
4. Detection range: 0.025 ng/mL-1.6 ng/mL. If the OD value of the sample to be tested is higher than 1.6 ng/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 0.025 ng/mL, the sample should be reported.

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 equipments. The following example data is for reference only.

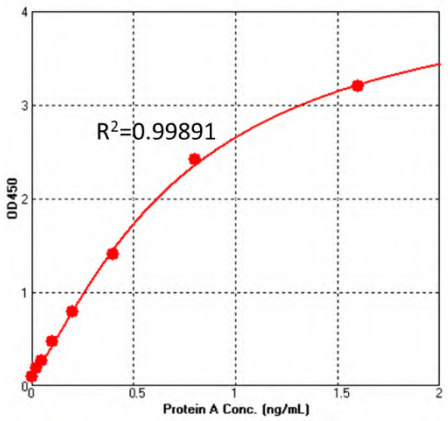
Standard curve of Recombinant Protein A:

Standard Num.	Concentration	OD_{450nm}
Standard 7	1.6 ng/mL	3.032
Standard 6	0.8 ng/mL	2.017
Standard 5	0.4 ng/mL	1.144
Standard 4	0.2 ng/mL	0.624
Standard 3	0.1 ng/mL	0.342
Standard 2	0.05 ng/mL	0.205
Standard 1	0.025 ng/mL	0.143
Standard 0	0 ng/mL	0.076



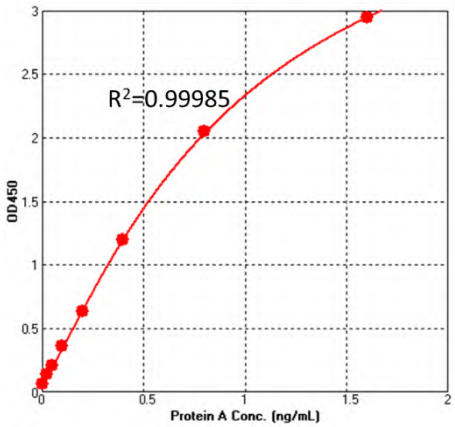
Standard curve of Alkali-tolerant Recombinant Protein A:

Standard Num.	Concentration	OD_{450nm}
Standard 7	1.6 ng/mL	3.197
Standard 6	0.8 ng/mL	2.415
Standard 5	0.4 ng/mL	1.4
Standard 4	0.2 ng/mL	0.795
Standard 3	0.1 ng/mL	0.474
Standard 2	0.05 ng/mL	0.267
Standard 1	0.025 ng/mL	0.182
Standard 0	0 ng/mL	0.092

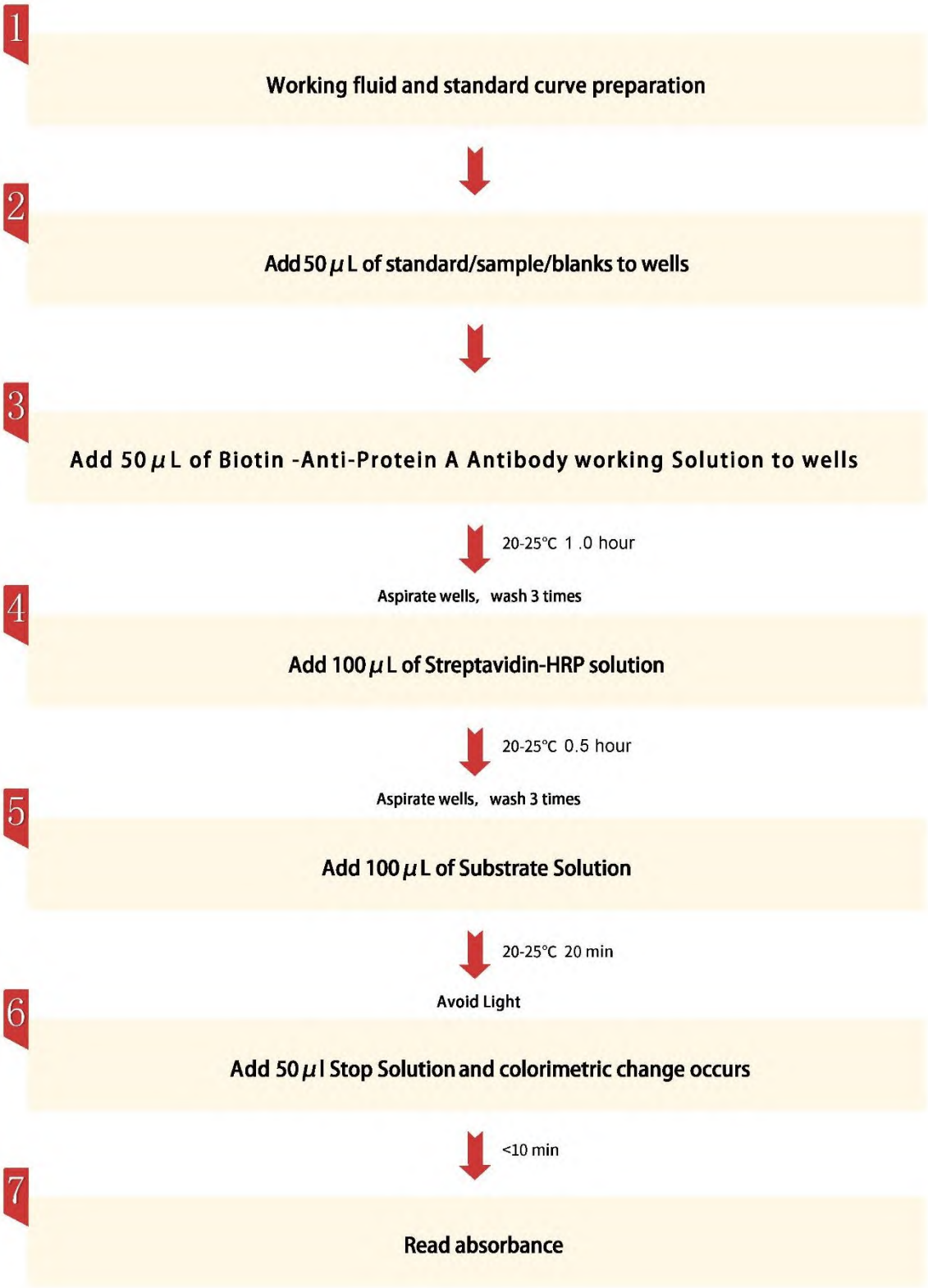


Standard curve of MaXtar® ARPA ligand Protein A (Bio-Link Co.):

Standard Num.	Concentration	OD_{450nm}
Standard 7	1.6 ng/mL	2.945
Standard 6	0.8 ng/mL	2.052
Standard 5	0.4 ng/mL	1.194
Standard 4	0.2 ng/mL	0.635
Standard 3	0.1 ng/mL	0.358
Standard 2	0.05 ng/mL	0.212
Standard 1	0.025 ng/mL	0.14
Standard 0	0 ng/mL	0.068



Quickguide


1
Working fluid and standard curve preparation
2
Add 50 μL of standard/sample/blanks to wells
3
Add 50 μL of Biotin -Anti-Protein A Antibody working Solution to wells
20-25℃ 1 .0 hour
Aspirate wells, wash 3 times
4
Add 100 μL of Streptavidin-HRP solution
20-25℃ 0.5 hour
Aspirate wells, wash 3 times
5
Add 100 μL of Substrate Solution
20-25℃ 20 min
Avoid Light
6
Add 50 μl Stop Solution and colorimetric change occurs
<10 min
7
Read absorbance

Performance Characteristics

Sensitivity

Protein A	Assay range (ng/mL)	Limit of quantification (LoQ*)
Recombinant Protein A	0.025-1.6 ng/mL	0.025 ng/mL
Alkali-Tolerant Recombinant Protein A	0.025-1.6 ng/mL	0.025 ng/mL
MaXtar® ARPA ligand Protein A (Bio-Link Co.)	0.025-1.6 ng/mL	0.025 ng/mL

Intra-Assay

	Recombinant Protein A			Alkali-Tolerant Recombinant Protein A			MaXtar® ARPA ligand Protein A Standard (Bio-Link Co.)		
Sample	1	2	3	1	2	3	1	2	3
Replicate Times	10	10	10	10	10	10	10	10	10
Mean (ng/mL)	1.5917	0.3027	0.0274	1.6154	0.284	0.0200	1.5885	0.2769	0.0236
CV (%)	2.39	3.35	8.88	1.71	2.75	8.90	1.78	0.98	6.92

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

Inter-Assay

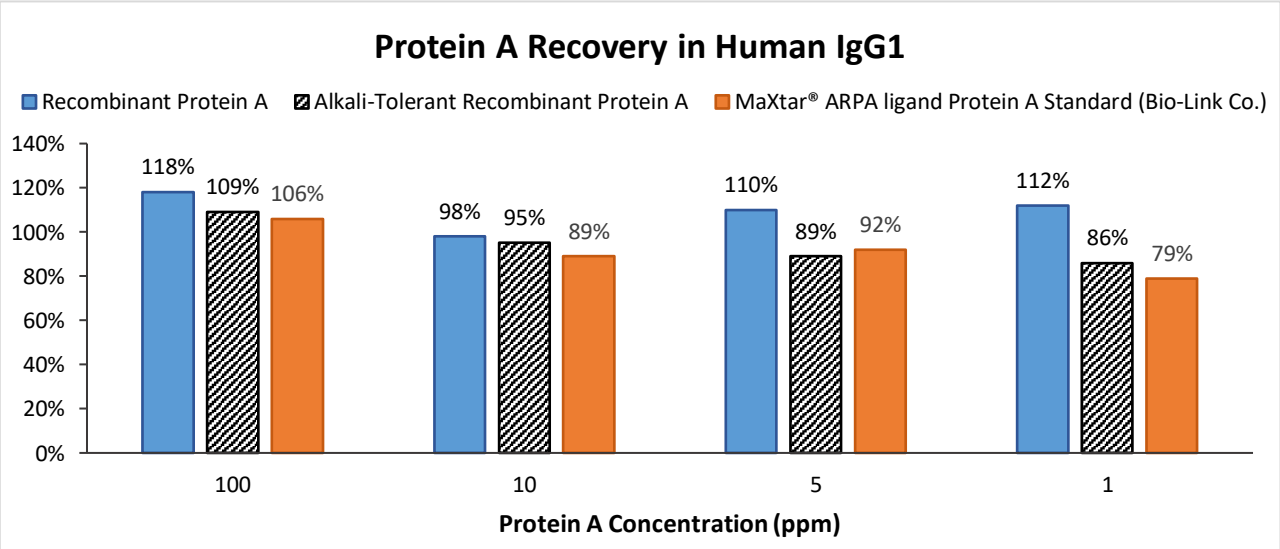
	Recombinant Protein A			Alkali-Tolerant Recombinant Protein A			MaXtar® ARPA ligand Protein A Standard (Bio-Link Co.)		
Sample	1	2	3	1	2	3	1	2	3
Replicate Times	10	10	10	10	10	10	10	10	10
Mean (ng/mL)	1.3138	0.2498	0.0259	1.5247	0.2655	0.0220	1.5732	0.2778	0.0246
CV (%)	11.12	9.10	7.65	6.74	11.11	13.88	6.67	8.11	10.20

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

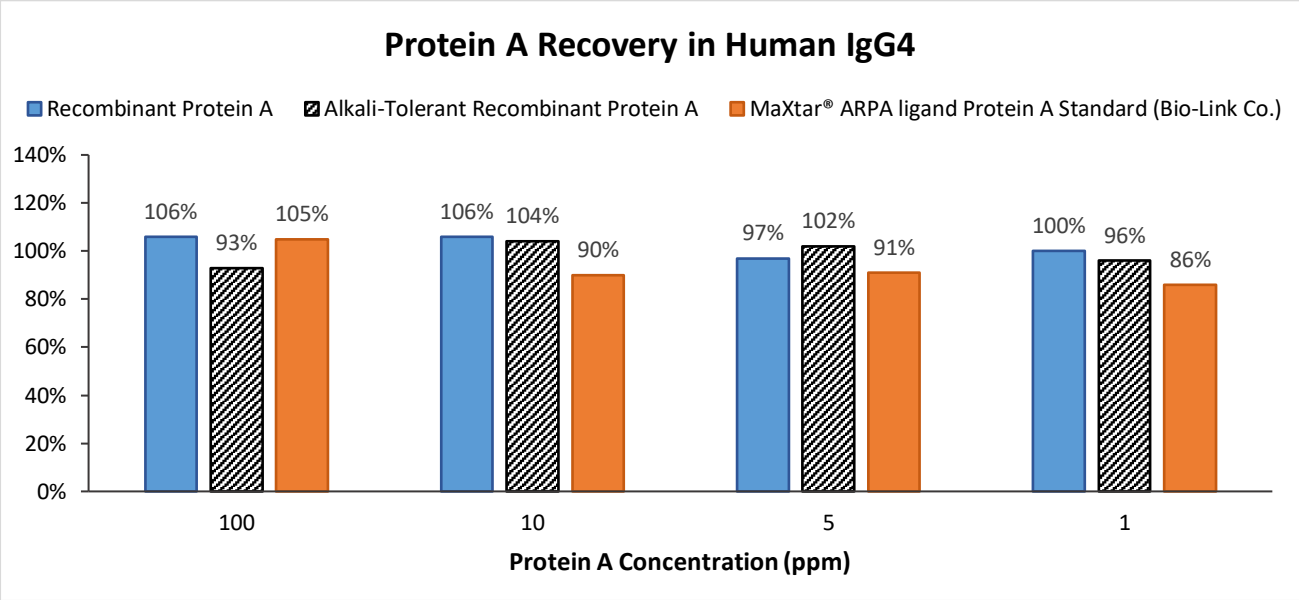
Recovery

Add different concentrations of Protein A to Human IgG1 (Bevacizumab) or Human IgG4 (Toripalimab), the final concentrations of Protein A were 100 ppm, 10 ppm, 5 ppm and 1 ppm respectively, the final concentration of Human IgG1 or Human IgG4 is 10 mg/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, Alkali-Tolerant Recombinant Protein A and MaXtar® ARPA ligand Protein A (Bio-Link Co.) to Human IgG1 (Bevacizumab):



Add Recombinant Protein A, Alkali-Tolerant Recombinant Protein A and MaXtar® ARPA ligand Protein A (Bio-Link Co.) to 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.)	
Matrix	**Recovery**	**Dilution Factor**	**Recovery**	**Dilution Factor**	**Recovery**	**Dilution Factor**
20mM L-histidine with 0.1% (w/v) PF68, pH6.0	92%	1	94%	1	102%	1
20mM L-histidine with 0.4% (w/v) Tween-80, pH6.0	98%	1	97%	1	110%	1
1*PBS, pH7.3	110%	2	107%	2	95%	2
1*PBS, pH7.3 with 11% Trehalose	104%	2	101%	2	89%	2
20mM L-histidine, pH6.0	105%	2	108%	2	101%	2
50mM Tris, 100mM Glycine, pH7.5	89%	2	100%	2	96%	2
100mM Tris, 20mM Sodium citrate, pH7.5	90%	2	95%	2	101%	2
20mM L-histidine 10% trehalose, pH6.0	113%	2	89%	2	102%	2
30% Trehalose	93%	2	96%	1	82%	2

Specificity

Host cell protein (HCP 500 ng/mL) and host cell DNA (HCD 0.5 ng/mL) 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 1.6ng/mL, 0.3ng/mL and 0.025ng/mL 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:

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
S2	S2-1	1	1.6	0	0
	S2-2	1	0.3	0	0
	S2-3	1	0.025	0	0
S3	S3-1	1	1.6	500	0.5
	S3-2	1	0.3	500	0.5
	S3-3	1	0.025	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)	Specificity of (Recombinant Protein A)	Specificity of (Alkali-Tolerant Recombinant Protein A)	Specificity of (MaXtar® ARPA ligand Protein A (Bio-Link Co.)
Bevacizumab	1	1.6	500	0.5	113.2%	116.9%	94.8%
Bevacizumab	1	0.3	500	0.5	106.3%	105.1%	114.9%
Bevacizumab	1	0.025	500	0.5	104.2%	116.2%	90.4%
Toripalimab	1	1.6	500	0.5	96.1%	105.7%	98.4%
Toripalimab	1	0.3	500	0.5	97.4%	96.4%	99.6%
Toripalimab	1	0.025	500	0.5	96.9%	94.6%	89.3%

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 cytokine 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