Protein A ELISA Kit

Cat. No. L00430

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I. Description

Protein A affinity chromatography is widely used for antibody purification. Protein A ligand leakage, even though covalently conjugated to the chromatography support, is a big problem for pharmaceutical industry. And it also probably causes false results in immunological assays if antibody product is contaminated with Protein A.

GenScript Protein A ELISA Kit is developed for the accurate quantification of native Protein A, recombinant Protein A variants and MabSelect SuRe™ (Alkali-tolerant recombinant protein A) in the presence or absence of antibodies. The kit offers a sensitive assay for users in pharmaceutical industry to measure mini amount of Protein A in therapeutic antibody products. Antibody suppliers can use the kit to evaluate Protein A containment level of the products. It also brings convenience to the manufacturers of Protein A affinity chromatography media in the process of monitoring leaching characteristics of the media under specific conditions.

This kit detects residual Protein A in a sandwich immunoassay. It utilizes two clones of Protein A monoclonal antibodies that bind Protein A at different domains. One monoclonal antibody is pre-coated on the polystyrene microtiter plate to capture Protein A in samples. Another monoclonal antibody with Biotin conjugated is then added to the plate to interact with Protein A. Subsequently horseradish peroxidase (HRP) labeled with streptavidin is added to bind the second antibody. The final step involves the addition of TMB Substrate. The color intensity is proportional to the amount of Protein A present in the test samples.

Sample process is crucial to the Protein A quantification assay. Leached Protein A can interact with Fc region of antibody, which negatively influence the accurate quantification of Protein A. Protein A ELISA Kit provides an optimized sample treatment protocol, thereby offering more reliable results.

II. Key Features

- Accurate quantification of 20 pg/ml Protein A contaminant or 50 pg/ml MabSelect SuRe™
- Broad-range detection of natural Protein A, recombinant Protein A variants and MabSelect SuRe™
- Time saving protocol - complete test in 2 hours or less
- Ready-to-use ELISA plate with fast & simple sample preparation
- Exceptional lot-to-lot consistency guaranteed by high-quality monoclonal antibodies
- Wide applications for the quantification of Protein A contaminant and leaching

III. Kit Contents

The kit provides all necessary reagents and buffers for Protein A quantification.

Components	Quantity
96-well plate coated with anti-Protein A mAb	1 plate (8 wells x 12 strips)
Biotin conjugated anti-Protein A mAb	0.5 ml
Streptavidin-HRP	12 ml
Recombinant Protein A Standard (10 µg/ml)	1 ml
MabSelect SuRe™ Protein A Standard (10 µg/ml)	1 ml
Antibody Dilution Buffer	15 ml
5 x Sample Dilution Solution	30 ml
20 × Wash Solution	40 ml
10% Tween 20	1 ml
One-Solution Microwell TMB Substrate	12 ml
Stop Solution	6 ml
Plate Sealer	2 pieces
User Manual	1 copy

IV. Storage

The reagents in the kit are stable for 12 months when stored at 2-8 °C.

V. Warnings and Precautions

- Reagents that contain preservatives may be toxic if ingested, inhaled, or spilled on skin.
- Avoid contact of skin, eyes, or clothing with *Stop Solution* or *One-Solution Microwell TMB Substrate*.
- *One-Solution Microwell TMB Substrate* is toxic if inhaled or swallowed. Avoid contact with skin. Keep container tightly closed. In case of an accident immediately seek medical advice.
  For more information, please visit the product webpage and MSDS sheet.

VI. Reagents/Equipments Required But Not Supplied

Distilled water
1 L graduated cylinder
Micropipettors P20, P200, P2000, and multi-channel pipettor
1.5 ml microcentrifuge tubes
Vortex mixer

Reagent reservoir dishes
Serological pipettes (5 ml, 10 ml)
1 L bottle top filter, 0.22 µm
ELISA plate reader, with wavelength capability at 450 nm
Timer
Microcentrifuge
Water bath

VII. Instruction for Use

Test Sample Preparation

It is important to dissociate and remove antibodies completely from the sample. Antibody associated with Protein A causes low recovery during the assay. The heating treatment could provide separated Protein A in the supernatant and denatured antibody in the precipitate. The highest antibody concentration of test sample should be diluted to 10 mg/ml or below because high antibody concentration interferes with assay result. The sample pH needs to be adjusted in the range between 6.0 and 7.5 because lower pH can also interfere with the assay results.

1. Add 500 µl of test sample containing Protein A into microcentrifuge tubes.
2. Add 5 µl of *10% Tween 20* into each sample to make its final concentration to 0.1% and mix thoroughly by vortexing.

Note: *The practical amounts of test sample can be adjusted depending on test requirement, and the working concentration of Tween 20 in sample solution should be 0.1%.*

3. Make a needle hole in the cap of each tube to release the air pressure during the heating procedure.
4. Place the tubes in a preheated 100°C boiling water bath for 10 minutes to dissociate Protein A-antibody complex.
5. Cool the tubes to room temperature and then centrifuge at 11 000 rpm for 5 minutes.
6. Transfer the supernatant containing Protein A to new tubes.

Note: *The supernatant will be used when preparing sample dilution in the assay procedure.*

7. Label the tubes containing samples to be tested and dilute the samples with 1 × *Sample Dilution Solution* if the Protein A concentration is high.

Note: *This protocol is suitable for all protein samples containing natural Protein A, recombinant Protein A variants and MabSelect SuRe™.*

Reagent Preparation

1 x Wash Solution: Dilute 40 ml of *20 × Wash Solution* with 760 ml of distilled or deionized water to make 800 ml of 1× Wash Solution. Filter 1 × Wash Solution through a 0.22 µm filter. If any precipitate forms in the *20 × Wash Solution* during storage, incubate the bottle in water bath (up to 50°C) with occasional mixing until all the precipitate disappears. Store 1× Wash Solution at 2-8°C.

1 x Sample Dilution Solution: Prepare desired volume of 1 x Sample Dilution Solution by adding 1 volume of *5 x Sample Dilution Solution* to 4 volume of distilled or deionized water. Filter the Solution through a 0.22 µm filter. If any precipitate forms in the *5 x Sample Dilution Solution* during storage, incubate the bottle in water bath (up to 50°C) with occasional mixing until all the precipitate disappears. Store 1 × Sample Dilution Solution at 2-8°C.

Biotin conjugated anti-Protein A mAb working solution Preparation

For the recombinant Protein A detection:

Make desired volume of Biotin conjugated anti-Protein A mAb working solution via diluting 1:150 of *Biotin conjugated anti-Protein A mAb* in *Antibody Dilution Buffer*.

For the MabSelect SuRe™ Protein A detection:

Make desired volume of Biotin conjugated anti-Protein A mAb working solution via diluting 1:50 of *Biotin conjugated anti-Protein A mAb* in *Antibody Dilution Buffer*.

Protein A Standards Preparation

For test sample containing native or recombinant Protein A, *Recombinant Protein A Standard (10 µg/ml)* should be used in preparation of the standard curve.

For test sample containing MabSelect SuRe™ Protein A, *MabSelect SuRe™ Protein A Standard (10 µg/ml)* should be used in preparation of the standard curve.

All reagents must be equilibrated to room temperature before use. Vortex the vial containing *Recombinant Protein A Standard* solution. Dilute proper amounts of *Recombinant Protein A Standard (10 µg/ml)* or *MabSelect SuRe™ Protein A Standard (10 µg/ml)* with 1 × Sample Dilution Solution to make 100.0 ng/ml before use. Store the diluted Protein A standard stock solution at 2-8°C.

Here an example is given to prepare the recombinant Protein A standards:

1. Label seven 1.5 ml microcentrifuge tubes with ''1.60 ng/ml'', ''0.80 ng/ml'', ''0.40 ng/ml'', ''0.20 ng/ml'', ''0.10 ng/ml'', ''0.05 ng/ml'', and ''0.00 ng/ml''.
2. Pipette 16 µl of prepared 100.0 ng/ml Recombinant Protein A standard solution and 984 µl of 1 × *Sample dilution Solution* into the tube labeled with ''1.60 ng/ml'' and vortex it.
3. Pipette 300 µl 1 × *Sample Dilution Solution* into rest empty tubes.
4. Pipette 300 µl 1.60 ng/ml Protein A solution to the tube labeled with ''0.80 ng/ml'' and vortex it to make the standard point of 0.80 ng/ml.
5. Similarly prepare series of rest standard points (0.40 ng/ml, 0.20 ng/ml, 0.10 ng/ml and 0.05 ng/ml).
6. Make a needle hole in the cap of each tube to release the air pressure during the heating procedure.
7. Place the seven tubes containing serial of Protein A concentration in a 100°C boiling water bath for 10 minutes.
8. Cool the tubes to room temperature and then centrifuge at 11 000 rpm for 5 minutes.

Note: *It is better that the heating treatment of test samples and Protein A standard points should be done at the same time.*

Here an example is given to prepare the MabSelect SuRe™ Protein A standards:

1. Label seven 1.5 ml microcentrifuge tubes with ''3.20 ng/ml'', ''1.60 ng/ml'', ''0.80 ng/ml'', ''0.40 ng/ml'', ''0.20 ng/ml'', ''0.10 ng/ml'', and ''0.00 ng/ml''.
2. Pipette 32 µl of prepared 100.0 ng/ml MabSelect SuRe™ Protein A standard and 968 µl of 1 × *Sample Dilution Solution* into the tube labeled with ''3.20 ng/ml'' and vortex it.
3. Pipette 300 µl 1 × *Sample Dilution Solution* into rest empty tubes.
4. Pipette 300 µl 3.20 ng/ml Protein A solution to the tube labeled with ''1.60 ng/ml'' and vortex it to make the standard point of 1.60 ng/ml.

5. Similarly prepare series of rest standard points (0.80 ng/ml, 0.40 ng/ml, 0.20 ng/ml, 0.10 ng/ml,).
6. Make a needle hole in the cap of each tube to release the air pressure during the heating procedure.
7. Place the seven tubes containing serial of Protein A concentration in a 100°C boiling water bath for 10 minutes.
8. Cool the tubes to room temperature and then centrifuge at 11 000 rpm for 5 minutes.
   Note: *It is better that the heating treatment of test samples and Protein A standard points should be done at the same time.*

Microtiter Plate Preparation

1. Design the experiment before removing the plate from protective pouch. Consider the appropriate amount of strip wells for standard curve and samples to test, sample dilution, duplicates vs triplicates, *etc*.
2. With gloved hands, tear the plate sealer from the plate and remove the strips that are not needed. Carefully wrap them in Parafilm and place them back into the foil pouch and store at 2-8°C.
3. Make sure the strips are properly snapped in the strip well holder. Cover the plate with plate sealer or Parafilm.

Setup of Protein A Standards and Test Sample Dilutions on Microtiter Plate

1. Transfer 100 µl of serial Protein A standard solution into columns 1-2 row A-G
2. Transfer 100 µl of serial test samples into columns 3-4 row A-D

Example for ELISA Plate Setup of Protein A standards and test sample dilutions

	Standard (ng/ml)		Test Sample									
	1 (duplicate 1)	2 (duplicate 2)	3 (duplicate 1)	4 (duplicate 2)	5	6	7	8	9	10	11	12
A	1.60	1.60	1:5	1:5								
B	0.80	0.80	1:10	1:10								
C	0.40	0.40	1:20	1:20								
D	0.20	0.20	1:40	1:40								
E	0.10	0.10										
F	0.05	0.05										
G	0.00	0.00										
H	Plate Blank											

Note: *To acquire an exact result, the sample dilution should be optimized in the process of detection.*

ELISA Test

This protocol is optimized for one 96-well plate. The volumes of the reagents can be scaled up or down according to the numbers of the strips or plates used. Samples should always be fully equilibrated to room temperature before dilution.

1. Pipette 100 µl of prepared Protein A standards and test samples into different wells as the procedures above. Cover the plate with Parafilm or plate sealer and incubate at 37°C for 30 minutes.
2. Aspirate the solution from the wells. With a wash bottle or automated plate-washing system, wash the plate with 1x Wash Solution and aspirate the fluid from the wells. Repeat the step four times.

Pound the plate vigorously on clean paper towels to remove excess liquid.

Note: *For each wash, 260 µl of 1× Wash Solution is suggested for each well.*

3. Briefly vortex bottle containing *Biotin conjugated anti-Protein A mAb* working solution. With a multi-channel pipettor, add 100 µl *of* solution into each well except 1H-2H (Plate Blanks). Cover the plate with Parafilm or plate sealer and incubate at 37°C for 30 minutes.
4. Repeat step 2.
5. Briefly vortex vials containing *Streptavidin-HRP* solution. With a multi-channel pipettor, add 100 µl of the solution into each well except 1H-2H (Plate Blanks). Cover the plate with Parafilm or plate sealer and incubate at 37°C for 10 minutes.
6. Repeat step 2.
7. With a multi-channel pipettor, add 100 µl of *One-Solution Microwell TMB Substrate* into each well including 1H-2H (Plate Blanks). And incubate at room temperature for 10～15 minutes.

Note: *The actual reaction time depends on the temperature. If temperature is low, reaction time should be extended. The ideal reaction temperature for optimum assay performance is 20°C-25 °C.*

8. With a multi-channel pipettor, Pipette 50 µl of *Stop Solution* into each well to stop the reaction. Read the plate at 450nm with an ELISA microwell plate reader.

Note: *The plate should be read within 30 minutes.*

9. Generate a standard curve by plotting the average absorbance on the vertical axis versus the corresponding Protein A standard concentration on the horizontal axis. The data can be linearized by using a linear regression analysis.

Suggested Calculation of Data

1. Calculate the mean absorbance value for the plate blank wells and subtract it from all remaining wells on the plate (including the 0 ng/ml standard). Determine the average absorbance values of each Standard concentration and tested samples.

Note: *Method of calculation for Standard Curve should be based on internal standards. Other curve fits may be used as deemed appropriate.*

2. Calculate the standard curve:

   Linear fit

   Plot each Standard Curve concentration (ng/ml Protein A) on the x-axis versus the corresponding mean absorbance value on the y-axis. Using linear regression, calculate the best fitting straight line through the points of the standard curve.

   Parameter fit

   The standard curve may be constructed using a 4 parameter logistic curve fitting program. Such a fit is the acknowledged reference model for sigmodial immunoassay data.

3. The regression line can be used to determine the Protein A concentration [PA] for the samples.

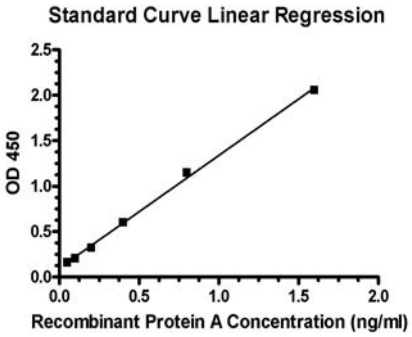
   [PA] x Sample Dilution = C (ng/ml)

   To determine the ng/mg (ppm) of Protein A in each sample well use the following formula.

$$\text{ng/mg} = \frac{\text{Mean Conc. [ng/ml]}}{\text{Conc. of undiluted antibody [mg/ml]}}$$

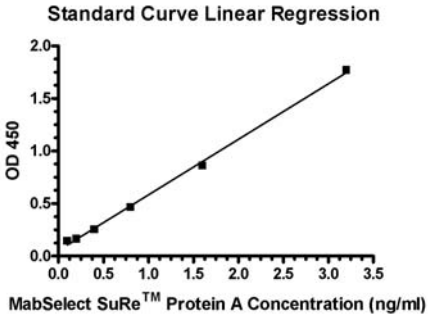
VIII. Typical Assay Data

Standard Curve of Recombinant Protein A



Conc. of Protein A (ng/ml)	OD 450			
	Duplicate 1	Duplicate 2	Average	Adjusted Average
1.60	2.077	2.034	2.056	2.002
0.80	1.154	1.140	1.147	1.093
0.40	0.591	0.616	0.604	0.550
0.20	0.318	0.329	0.324	0.270
0.10	0.202	0.207	0.205	0.151
0.05	0.161	0.162	0.162	0.108
0.00	0.084	0.090	0.087	0.033
Blank	0.052	0.055	0.054	

Standard Curve of MabSelect SuRe™ Protein A



Conc. of Protein A (ng/ml)	OD 450			
	Duplicate 1	Duplicate 2	Average	Adjusted Average
3.20	1.711	1.829	1.770	1.717
1.60	0.849	0.871	0.860	0.807
0.80	0.461	0.468	0.465	0.412
0.40	0.247	0.257	0.252	0.199
0.20	0.163	0.164	0.164	0.111
0.10	0.141	0.149	0.145	0.092
0.00	0.077	0.076	0.077	0.024
Blank	0.052	0.054	0.053	

IX. Precision

Precision is defined as the percent coefficient of variation (% CV). The data below shows both intra and inter-assay coefficients of variation for 3 control samples in the low, mid and high concentration range of the assay.

Intra-assay Precision: CV<5% and Inter-assay Precision: CV<10%

intra-assay			inter-assay		
# of tests	Meaning/ml	CV%	# of assays	Meaning/ml	CV%
12	1.60	1.60	8	1.60	3.70
12	0.40	4.05	8	0.40	5.42
12	0.10	4.56	8	0.10	7.59

X. Specificity

This ELISA Kit can detect following Protein A ligands:

- Native Protein A, such as ligands from GE Healthcare HiTrap™ Protein A HP Columns (Cat. No. 17-0402-03)
- Recombinant Protein A variants, such as ligands from GE Healthcare HiTrap™ rProtein A FF (Cat. No. 17-5079-02), GenScript Protein A Resin (Cat. No. L00210) and Ultra Protein A Resin (Cat. No. L00400).
- GE Healthcare MabSelect™ chromatography media, such as GE Healthcare MabSelect SuRe™ (Cat. No. 17-5438-01)

For the performance of the kit with special Protein A, please contact our Technical Support.

XI. Sensitivity

The lower limit of detection (LOD) is defined as the concentration corresponding to a signal three standard deviations above the mean of the zero standard. The lower limit of quantitation (LOQ) is defined as the first dosed standard.

Sample	LOD	LOQ
Recombinant Protein A	20 pg/ml	50 pg/ml
MabSelect SuRe™ Protein A	50 pg/ml	100 pg/ml

XII. Recovery

Various buffer matrixes have been evaluated by spiking known amounts of Protein A. Users should validate that their sample matrixes and the kit yield accurate recovery. This experiment can be performed by spiking the standard provided with this kit, into the sample. The added spike and recovery should be within allowable limit 85% to 115%.

Sample Matrix	Protein A Added (ng/ml)	% Recovery
6.20 mg/ml human IgG	1.50	85.01
1.19 mg/ml customer Ab	1.00	98.07
1.13 mg/ml human IgG	1.20	108.96
1.00 mg/ml human IgG	2.50	100.01

XIII. Troubleshooting

Problem	Probable Cause	Solution
Poor Precision	Inadequate washing / aspiration of wells	Check function of washing system
	Inadequate mixing of reagents	Ensure adequate mixing
	Imprecise / inaccurate pipetting	Check / calibrate pipette
Poor Standard Curve	Improper standard handling / dilution	Ensure correct preparation of standards
	Incomplete washing / aspiration of wells	Check function of washing system
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
Poor Signal	Inadequate reagent volumes added to wells	Check/ calibrate pipettes
	Incorrect incubation time/ temperature	Ensure sufficient incubation time/reagents warmed to RT
	Incorrect antibody titration	Check detection Ab and Working Detection reagent preparation
High background	Inadequate washing	Check washing system
	Contaminated Wash Solution	Make fresh Wash Solution

XIV. Related Products

- Ultra Protein A Resin L00400
- Protein A Resin L00210
- Streptavidin-HRP M00091
- Protein A Antibody, mAb, Mouse A01778
- Protein A Antibody [Biotin], mAb, Mouse A01779
- Protein A Antibody, pAb, Chicken A00728
- Protein A Antibody [HRP], pAb, Chicken A00729

For laboratory or further manufacturing use only.

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