Protein A ELISA Kit PLUS

Cat. No. Protein A-037KIT **Lot. No.** (See product label)

SPECIFICATION

Product Overview	In the purification process of antibody drugs, if the shed protein A is mixed into the antibody, it will lead to the contamination of antibody drugs. This kit is used to quantitatively analyze protein A impurities in biological drugs, and can detect both natural protein A and recombinant protein A. The kit uses a Double antibody sandwich method to determine the amount of protein A in the sample. Compared with traditional methods, this kit is simple and time-consuming to detect protein A isolated from IgG without a sample pretreatment step. Lowest limit of detection: The lowest limit of detection (LOD) for the assay kit reference material should be ≤5.2 pg/mL. Accuracy: The relative deviation for the assay kit reference material should be within ±10%. Linear: The correlation coefficient (r) of the kit should be ≥ 0.9900 in the measurement range of [5.2-1600] pg/mL. Precision: The coefficient of variation (CV) for the assay kit reference material should be ≤ 10%. Recovery rate: Different concentrations of Protein A added to the antibody (1 mg/mL) were detected and the recovery rate was calculated. The recovery rate of this kit was in the range of 85.0% to 115.0%.
Description	Protein A, a cell wall protein isolated from Staphylococcus aureus, binds to the Fc segment of most types of IgG (except IgG3) for immunoassay and IgG purification. In the purification process of monoclonal antibody drugs, protein A-based affinity chromatography is one of the widely used purification methods. However, if the shed Protein A is mixed into the antibody, it will lead to contamination of the antibody drug. This kit can detect both natural protein A and recombinant protein A, and is suitable for quantitative analysis of protein A impurities in biopharmaceuticals. In general, protein A forms a complex with monoclonal antibody in the sample, which affects the accurate determination of protein A. Compared with traditional methods, this kit can

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	detect protein A isolated from IgG without sample pretreatment, which makes the experiment simple and easy to operate, and takes relatively little time.
Form	1. All components of the kit should be complete and intact, and there should be no leakage of liquid components. 2. The liquid components should be clear, without precipitation or flocculent substances. 3. The packaging labels should be clear and easy to identify.
Kit Compoents	This kit provides all the reagents required for the detection of Protein A. Reagent A: 1. Recombinant Protein A Calibrator (2 μg/mL); 2. MabSelect SuReTM Protein A Calibrator (2 μg/mL) Reagent B: Diluent Reagent C: Biotin-labeled detection antibody Reagent D: Streptavidin-HRP complex Reagent E: Wash Buffer (10×) Reagent F: 1. TMB Chromogen Solution (Component A); 2. TMB Chromogen Solution (Component B) Reagent G: Stop Solution Component H: Pre-coated microplate strips Component I: Plate sealers
Essentials not provided	1. A microplate reader with dual-wavelength detection at 450nm and 630nm. (If the microplate reader does not support dual-wavelength analysis, you can measure the absorbance at a single-wavelength of 450 nm only.) 2. Pipettes: 5-200 μL. 3. Multi-channel pipette: 100 μL. 4. Distilled or purified water. 5. 500mL container for dilution of Wash Buffer.
Principle	This kit uses the double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) technique to determine the content of protein A in the samples. Experimental principle. Calibrators and samples to be tested are added to the microplate, and unbound substances are washed away. At this point, a "Coated Antibody - Protein A" complex is formed on the microplate. Subsequently, a biotin - labeled anti-protein A detection antibody is added to form a "Coated Antibody-Protein A-Biotin-Labeled Detection Antibody" complex. After incubation at room temperature for 1 hour, unbound substances are washed away. Then, a streptavidin-horseradish peroxidase

	(SA-HRP) complex is added and incubated at room temperature for 30 minutes, thus forming a "Coated Antibody-Protein A-Biotin-Labeled Detection Antibody-SA-HRP" complex on the microplate. After washing away the unbound substances, TMB chromogenic solution is added. The chromogenic solution is catalyzed by horseradish peroxidase to turn blue, and then turns into the final yellow under the action of an acidic stop solution. The absorbance value (O.D. value) of each well is read by a microplate reader at a dual-wavelength of 450nm/630nm, and the content of protein A in the samples to be tested is calculated through a standard curve.
Advantages	1. Versatility: adaptable to a wide range of fillers; 2. Scientific and efficient sample processing method: Sample does not need boiling, heating, centrifugation and other complicated steps; 3. Simple and fast: Test completed in 3 hours; 4. High sensitivity and wide linear range: The minimum detection limit is 5.2 pg/mL and the linear range is 5.2-1600 pg/mL; 5. Good spiking recoveries: The kit is suitable for a wide range of protein A filler ligands.
Usage	Reagent preparation 1. This reagent is sensitive to moisture. Before opening the bottles, allow all the component vials to fully equilibrate to room temperature to prevent moisture condensation inside the containers. 2. Preparation of Wash Buffer: Dilute Reagent E Wash Buffer (10×) 10-fold (1:9) with distilled water or purified water to prepare the wash buffer for the experiment. For long-term use, it can be stored at 2-8 centigrade, with a validity period of 3 months. 3. Set the microplate reader to read at dual wavelengths of 450nm/630nm. 4. Preparation of TMB Chromogen Solution: The TMB Chromogen Solution consists of two parts: Component A (including H2O2) and Component B (including TMB), which are packaged in separate vials. Mix them at a ratio of 1:1 immediately before use. Use the prepared solution right away. 5. Preparation of calibrators: When testing samples containing natural or recombinant Protein A, please refer to the following dilution procedure to prepare a standard curve: 1600.00 pg/mL, 800.00 pg/mL, 400.00 pg/mL, 160.00 pg/mL, 64.00 pg/mL, 25.60 pg/mL, 10.24 pg/mL, 0.00 pg/mL. A corresponding standard curve should be

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	prepared for each experiment, and the prepared calibrators should be used within 4 hours as much as possible. 6. Sample Preparation: Generally, Protein A forms a complex with the antibodies in the sample to be tested, and high concentrations of IgG can affect the results, thus affecting the accuracy determination of the Protein A. Therefore, before determining Protein A, the sample must be diluted with Reagent B at a dilution ratio of at least 1:1. It is recommended to dilute the sample to a protein concentration of 0.05-4.0 mg/mL, and ensure that the concentration of Protein A in the sample is within the linear range of detection. The volume of the diluted sample should be no less than 150μ. Note: This kit can resist interference from IgG or proteins up to a concentration of 5 mg/mL.
Applications	This kit is intended for scientific research and production process control, and cannot be used for medical diagnosis in humans and animals.
Note	1. This kit is suitable for use by qualified technicians. 2. It is necessary to test the sample's dilution concentration. The sample should be diluted more than 10-fold or gradient-diluted to several different concentrations for testing. 3. Remove the kit from refrigeration and equilibrate at room temperature for 20 minutes before use. 4. To avoid cross-contamination of reagents, pipette tips and plates, disposable pipette tips and reagent containers should be used. Do not re-pour unused reagents back into reagent bottles or tubes, and be careful not to mix the caps of different reagent bottles and cause cross-contamination. 5. Sampling: It is best to control the time for each sample addition within 5 minutes, and is recommended to set multiple holes. If the number of samples is large, it is recommended to use the row of guns to add samples. 6. Please prepare the standard curve (duplicate wells) in each assay. For all assay steps, maintain a consistent time interval between wells to ensure the incubation time. All steps should be completed as quickly as possible. 7. Incubation: In order to prevent sample evaporation, the laboratory must use a plate sealing film, the next step should be performed as soon as possible after washing the plate, and the incubation time and temperature should be strictly observed. It is recommended

to shake the microplate at 500 rpm during the 3 incubations before adding TMB Chromogen Solution. Static incubation may cause the overall OD value to be lower by about 30%, but it will not affect the results of the assay. 8. Wash the plate: The wash buffer remaining in the wells during the washing process should be patted dry on absorbent paper or a dust-free dry cloth. Do not place filter paper directly into the reaction wells to absorb water. Under normal circumstances, the detected O.D. value at the concentration point of Calibrator A (0.00 pg/mL) should be less than 0.1. If it is higher than this value, you can consider increasing the number of washing repetitions. 9. Color development: Please observe the color change of the reaction wells regularly (e.g. every 2 minutes). If the gradient is already obvious, you can add the stop solution to terminate the reaction in advance to avoid the color being too deep; when the room temperature is low, you need to extend the color development time appropriately, and it is best to use the OD at the highest calibration concentration of about 2.0-2.5. Due to the operating habits of the experimenters and the experimental environment, the maximum OD value of the kit may vary, which is a normal phenomenon, and the TMB Chromogen Solution should be stored away from light. 10. Termination reaction: Stop solution is acidic and requires extra care during handling to prevent contact with skin and eyes. 11. Plate reading: Before reading with the microplate reader, gently wipe the bottom of the microplate with a clean paper towel to remove residual liquid and fingerprints, so as not to affect the reading of the microplate reader. The OD630nm is used as the calibration wavelength during the reading process, if there is a difference between the instruments, 650nm can also be used instead of 630nm. 12. You can use the same protein A standard as the calibrator according to your needs, but you need to dilute the high-concentration protein A to 3 ng/mL with Reagent B, and prepare each concentration point according to the calibrator preparation table and the illustration.

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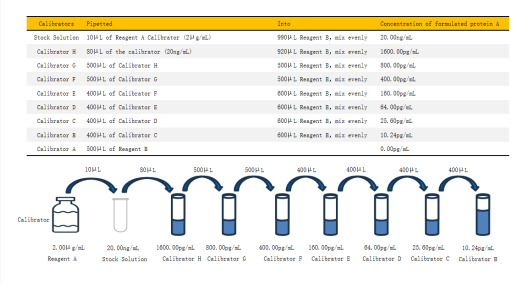
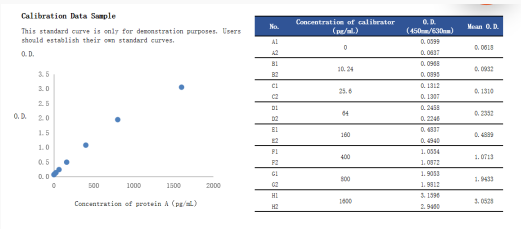


Calibrators	Pipetted	Into	Concentration of formulated protein A
Stock Solution	10μL of Reagent A Calibrator (2μg/mL)	990μL Reagent B, mix evenly	20.00μg/mL
Calibrator H	80μL of the calibrator (20ng/mL)	920μL Reagent B, mix evenly	1600.00pg/mL
Calibrator G	500μL of Calibrator H	500μL Reagent B, mix evenly	800.00pg/mL
Calibrator F	500μL of Calibrator G	500μL Reagent B, mix evenly	400.00pg/mL
Calibrator E	400μL of Calibrator F	600μL Reagent B, mix evenly	160.00pg/mL
Calibrator D	400μL of Calibrator E	600μL Reagent B, mix evenly	64.00pg/mL
Calibrator C	400μL of Calibrator D	600μL Reagent B, mix evenly	25.60pg/mL
Calibrator B	400μL of Calibrator C	600μL Reagent B, mix evenly	10.24pg/mL
Calibrator A	500μL of Reagent B		0.00pg/mL

Calibration Data Sample



Calibration Data Sample

This standard curve is only for demonstration purposes. Users should establish their own standard curves.

O.D.

No.	Concentration of calibrator (pg/mL)	O.D. (450nm/630nm)	Mean O.D.
A1	0	0.0399	0.0618
A2		0.0837	
B1	10.24	0.0968	0.0932
B2		0.0895	
C1	25.6	0.1312	0.1310
C2		0.1307	
D1	64	0.2458	0.2332
D2		0.2206	
E1	160	0.4037	0.4089
E2		0.4140	
F1	400	1.0554	1.0713
F2		1.0872	
G1	800	1.9033	1.9433
G2		1.9812	
H1	1600	3.1396	3.0528
H2		2.9660	

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