

(For Research Use Only. Not For Use In Diagnostic Procedures!)

**FineTest®**

## **Protein A ELISA Kit**

Catalogue No.: EU2662

Revision: V4.0

Size: 96T

Please do not mix and use reagents from different kits or different batches. Otherwise, it might not work properly.

Please read the manual carefully before use. Feel free to contact us if you have any questions.

Email            [fine@fn-test.com](mailto:fine@fn-test.com)

Website        <https://www.fn-test.com/>

Please provide the batch number (see kit label) for more rapid response and services.

It's strongly recommended to use this kit within the expiry date printed on the kit label.

**Reactivity:** Universal

**Range:** 78.125-5000pg/ml

**Sensitivity:** 46.875pg/ml

**Application:** This Protein A ELISA kit is used for quantitative detection of residual Protein A in purified IgG antibody. It can be only used for scientific research and production, but not for the diagnosis and treatment of human or animal diseases. This kit provides an acidifying method for separating protein A from IgG. Before sample detection, Micro-volume UV Spectrophotometer or BCA method is required to detect concentration. It's suggested to regulate final IgG concentration up to 2mg/mL.

### Principle of the Assay

This kit used a double antibody sandwich ELISA method, and the experiment lasted for 2 hours. The microplate in the kit was pre-coated with an antibody against Protein A. The biotin-detection antibody was added to the well plate. Then the pretreated standard and the moderately diluted sample to be tested were added to the corresponding wells. The unbound components were washed off after incubation. HRP-streptavidin (HRP-SA) was added. Then the unbound components were washed away and TMB chromogenic substrate was added, which was catalyzed by horseradish peroxidase (HRP) to appear blue and turned yellow after adding the stop solution. OD values were measured at 450 nm using a microplate reader. The concentration of Protein A in the samples was calculated by plotting a standard curve. The concentration of the target substance is proportional to the OD450 value.

### Kit Components and Storage

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below:

No.	Item	Size(96T)	Storage Condition for Opened Kit
E001	ELISA Microplate(Dismountable)	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 12months at -20°C.
E002	Lyophilized Standard	2 vial	2-8°C (Avoid Direct Light)
E003	Biotin-labeled Antibody (Ready to use)	10 ml	
E034	HRP-Streptavidin Conjugate (Ready to use)	10 ml	
E024	TMB Substrate	10 ml	
E035	Acidifying reagent	6 ml	
E039	Sample Dilution Buffer	30 ml	2-8°C
E026	Stop Solution	10 ml	
E038	Wash Buffer(25X)	30 ml	
E006	Plate Sealer	5 pieces	
E007	Product Description	1 copy	

Notes: The volume of liquid reagent provided in the bottle is slightly more than the labelled size. Please suck the exact volume with pipette and dilute at the relevant ratio.

### Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
3. Precision single (0.5-10 $\mu$ L, 5-50 $\mu$ L, 20-200 $\mu$ L, 200-1000 $\mu$ L) and multi-channel pipette with disposable tips (calibration is required before use.)
4. Sterile tubes and Eppendorf tubes with disposable tips
5. Absorbent paper and loading slot
6. Deionized or distilled water

### Reagent Preparation and Storage

Take the ELISA kit from the fridge around 20 minutes earlier and equilibrate to room temperature(18-25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

#### 1. Wash Buffer

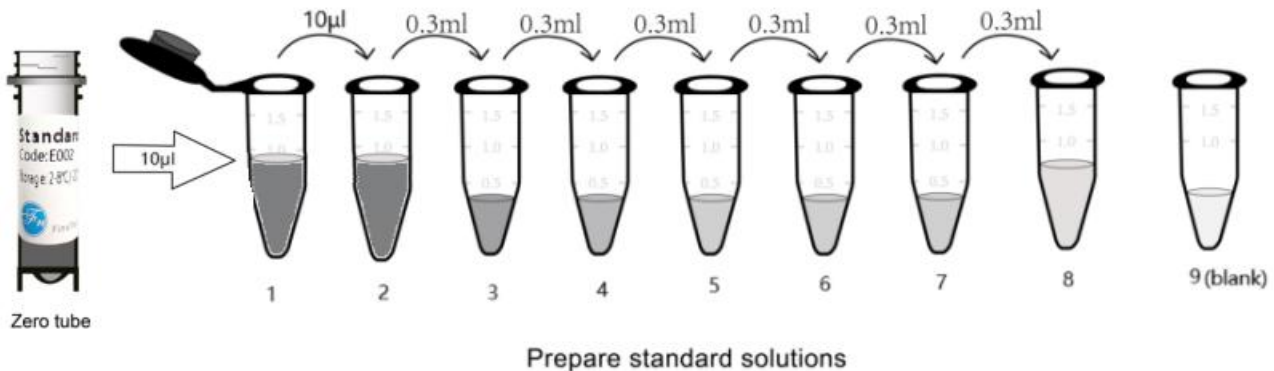
Dilute 30ml concentrated wash buffer to 750ml wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18M $\Omega$ .) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8°C.

Crystals formed in the concentrated wash buffer can be heated by water bath at 40 °C till complete dissolution. (Heating temperature should be below 50°C.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8°C within 48h.

#### 2. Standards

2.1. **Standard in the zero tube:** centrifuge the lyophilized standard tube at 10000 $\times$ g for 1min. Add 100 $\mu$ l sample dilution buffer into the lyophilized standard tube. Tighten the tube cap and let it stand for 2min at room temperature. Gently shake several times to mix well (Alternatively, add 100 $\mu$ l sample dilution buffer and let it stand for 1-2min. Then mix for 3-5s with low-speed vortex mixer.). Centrifuge at 1000 $\times$ g for 1min at low speed. Collect the liquid to the bottom of the tube. Then, the concentration of standard in the zero tube is 50  $\mu$ g/ml.

2.2. Gradient dilution: Nine EP tubes were taken and labeled as tubes 1-9 respectively. 990 $\mu$ L of sample diluent was added to tubes 1 and 2, and 300 $\mu$ l of sample diluent was added to tubes 3-9. 10 $\mu$ l of the concentrated standard was added to tube 1, vortexed and mixed, and then 10 $\mu$ l was added to tube 2 and vortexed and mixed. Then, 300 $\mu$ l of liquid in tube 2 was added to tube 3 and vortexed to mix, and 300 $\mu$ l of liquid in tube 3 was added to tube 4 and vortexed to mix until tube 8. Note that EP tube 9 only contains the sample diluent. At this time, the concentration of standard material in 8 EP tubes from tube 2 to tube 9 was 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312.5pg/ml, 156.25pg/ml, 78.125pg/ml and 0pg/ml, respectively. Once the standards and samples are ready, the sample treatment procedure begins.



**Notes:** Store the dissolved standard in the zero tube at 2-8°C, and use within 12h. Standard working solutions diluted in other gradient should be used within 2h.

### 3. Sample Dilution and Pretreatment

- 3.1. Sample Dilution: If the sample needs to be diluted, please use the sample diluent in the kit to dilute in EP tube.
- 3.2. Add 100µl of pilot sample to each sample processing tube.
- 3.3. Add 100µl of gradient standard to each standard processing tube (tube 2 to tube 9).
- 3.4. Add 50µl of acidifying solution to each well, and use the pipettor to blow and mix at least 15 times (if EP tube is used, EP tube can be vortexed for 5 seconds).
- 3.5. Place at room temperature for 5 min (to separate protein A and antibody in the sample by acidification). Then, perform the assay immediately.

#### Assay Procedure Summary

**Step 1:** Add 100ul of biotin-antibody working solution into each well, and then add 25µl of acidified standards, samples, quality controls, and blanks to the corresponding wells (**Change tips for different samples and standards**). Seal the plate and gently shake the microplate for 10s. Statically incubate for 60 min at room temperature(18-25°C) in the dark.

Washing: Wash 3 times. Immerse for 1min each time. Tap the microplate on the absorbent paper.

**Step 2:** Add 100ul HRP-streptavidin (HRP-SA) working solution, seal the plate and statically incubate for 10 minutes at room temperature(18-25°C) in the dark.

Washing: Wash 5 times. Immerse for 1min each time. Tap the microplate on the absorbent paper.

**Step 3:** 90ul of TMB chromogenic substrate was added. Seal the plate and statically incubate for 10-20 minutes at room temperature(18-25°C) in the dark. (Accurate TMB visualization control is required.).

**Step 4:** Add 50ul of stop solution. Read at 450nm immediately and calculate.

## Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors.
2. **Sample Loading:** Add 100ul biotin-labeled antibody working solution into each well, then add 25ul acidified gradient standard to the standard well (tube 2 to tube 9). Add 25ul moderately diluted and acidified tested sample to the sample well (**Change tips for different samples and standards**). Seal the plate and gently shake the microplate for 10s. Statically incubate for 60 minutes at room temperature(18-25°C) in the dark.
3. **Wash Three Times:** Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 300-350ul wash buffer into each well. Immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.
4. **Addition of HRP-streptavidin (HRP-SA):** Add 100ul ready-to-use HRP-streptavidin (HRP-SA) into each well. Seal the plate and Statically incubate for 10 minutes at room temperature(18-25°C) in the dark.
5. **Wash Five Times:** Remove the cover, and then wash the plate five times with wash buffer. Read washing method in step 3.
6. **Addition of TMB Substrate:** Add 90ul TMB Substrate into each well, seal the plate and statically incubate for 10-20 minutes at room temperature(18-25°C) in the dark. Run the microplate reader and preheat for 15min.  
  
(Notes: Please do not use the loading slot preparing for HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent blue gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable. Please refer to TMB color rendering control in page 2 or QR code for detail.)
7. **Addition of Stop Solution:** Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow from blue immediately. The order for adding stop solution and TMB substrate solution is the same.
8. **OD Measurement:** Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a configuration of wavelength correction, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

## Calculation of Results

1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.
2. Create a four parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, [Curve Expert 1.3](#) or [1.4](#) available in FineTest website).

3. Calculate the sample concentration by substituting OD450 value into the standard curve. Recovery validation is specified in page 7. Customers can choose the relevant recovery to recalculate the result and determine the final Protein A concentration according to their own antibody concentrations.

Notes: **Diluted samples should be multiplied by the relevant dilution ratio.**

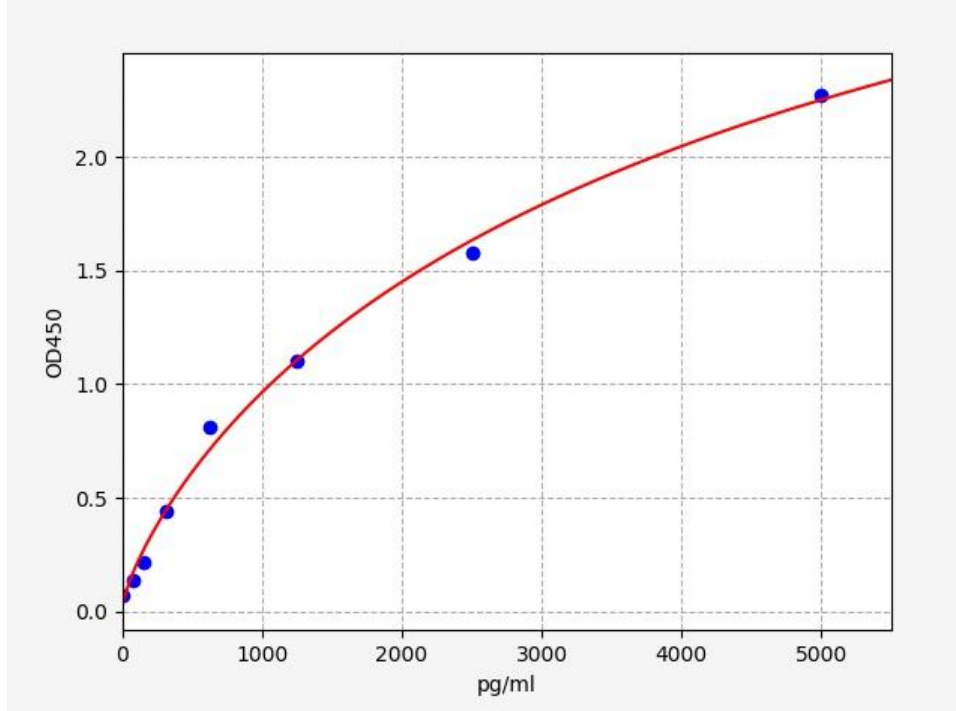
#### Notes for Plotting Different Standard Curves

1. Linear Curve: One coordinate axis stands for the antigen concentration. Another stands for OD450 value.  $R^2$  value is used to determine fitting. Higher than 0.99 shows the better fitting. However, linear curve usually compresses data point at the bottom of the curve, resulting in the inaccurate calculated results.
2. Semilogarithmic Curve: Helps to counteract bottom compression caused by linear curve. Semilogarithmic curve utilizes the relationship between logarithm and reading of concentration. This method usually can get S-shaped curve with uniformly distributed data point.
3. Logarithmic/double logarithmic Curve: Provide better linearity for low to medium concentration range. But high concentration range easily loses linearity.
4. 4PL or 5PL Curve: This method is more complex, considering other parameters (e.g. maximum and minimum value). Thus, more complex calculation is required. 4PL supposes symmetry around inflection point. 5PL considers the asymmetric situation which is more suitable for immunoassay analysis. If your software supports, both 4-PL and 5-PL are suitable for correcting most ELISA standard curves.

### Typical Data & Standard Curve

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(pg/ml)	OD-1	OD-2	Average	Corrected
0	0.07	0.072	0.071	0
78.125	0.136	0.14	0.138	0.067
156.25	0.214	0.22	0.217	0.146
312.5	0.433	0.445	0.439	0.368
625	0.799	0.823	0.811	0.74
1250	1.084	1.116	1.1	1.029
2500	1.556	1.602	1.579	1.508
5000	2.24	2.304	2.272	2.201



### Recovery Validation

PBS (pH7.2) system is common for antibody storage and applications. Sample dilution buffer(E039) in this kit is 10mM PBS (pH7.2-7.4). Customers can also prepare by themselves.

Recovery test results are listed below using 10mM PBS buffer (PH7.2-7.4) with different concentrations of IgG as sample matrix.

Sample Type		Recovery Range (%)	Average (%)
10mM PBS (PH7.2-7.4)	IgG content =1mg/ml	94-102	97
	IgG content =0.5mg/ml	95-103	98
	IgG content =0.1mg/ml	95-105	99

Customers can select the recovery relevant to their own antibody concentration to determine the final Protein A concentration via recalculating the result. Alternatively, refer to methods above to validate recovery.

### Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on the same plate, Intra-assay CV<8%.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates. Inter-assay CV<10%.

### Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

ELISA kit(n=5)	37°C for 1 month	2-8°C for 6 months	2-8°C for 12 months
Average (%)	80	95-100	85-98

### Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.
2. During the ELISA kit development, some endogenous interferons (not all) in the biological sample have been removed or decreased.
3. The final assay result is related to the validity of reagents, experimental operation and environment. FineTest is only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.
4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.
5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.
6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.

7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.

8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.

9. This kit allows for research use only. For IVD or other purposes, FineTest is not responsible for relevant consequences and doesn't bear any legal liability.