Technical Manual

Rabbit OVA sIgE (Ovalbumin Specific IgE) ELISA Kit

- Catalogue Code: AEFI01414
- Capture ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

OVA sIgE ELISA Kit, Ovalbumin Specific IgE ELISA Kit

Detection method:

Capture ELISA

Sample Type:

{Samples type}

Reactivity:

Rabbit

Range:

1.563-100ng/ml

Sensitivity:

0.938ng/ml

Storage:

2-8°C for 6 months (sealed), please do not freeze!

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this unopened ELISA Kit at 4°C for 6 months. For opened kits, store individual components as described in the Kit Contents section. Date of expiration will be on the ELISA Box label.

3. Description and Principle

Assay Genie ELISA Kits are designed for the precise measurement of analytes in a wide variety of sample types. In response to the increasing demand for high-quality, consistent data from today's scientists, we have developed a range of sensitive, fast, and reliable ELISA kits that meet and exceed these expectations.

The Assay Genie Capture ELISA kit allows for the quantitative measurement of specific antibodies against Rabbit OVA sIgE in the following samples: Rabbit Serum, Plasma, Cell culture supernatant, Cell or tissue lysate, Other liquid samples.

How do our ELISA kits work?

This kit is based on Capture ELISA technology. Anti-Rabbit- antibodies are pre-coated onto 96-well plates. The general antibodies present in the sample are captured. Next, a Rabbit OVA sIgE conjugated to horseradish peroxidase (HRP) is added to detect the target-specific antibodies present in the samples. HRP catalyses the TMB substrate to produce a blue colour product that is then changed to yellow after adding the acidic stop solution. The concentration of specific antibodies can be calculated by reading the O.D. absorbance at 450nm in a microplate reader and comparing the results with the standard curve. A reference wavelength of 650nm can be used.

4. Kit Contents

The sealed kit can be stored at 2-8 °C. The storage condition for opened kit is specified in the table below. Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (Dismountable)	8×6	8×12	Put the rest of the strips into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C or for 6 months at -20°C
2	Lyophilized Standard	1vial	2 vial	Put the rest of the strips into a sealed foil bag with the desiccant. Store for 1 month at 2-8°C or for 6 months at -20°C
3	HRP-labeled Antigen (Concentrated, 100X)	60ul	120 ul	2-8°C (Avoid Direct Light)
4	TMB Substrate	5ml	10 ml	
5	Sample Dilution Buffer	10ml	20 ml	2-8°C
6	Antigen Dilution Buffer	5ml	10 ml	
7	Stop Solution	5ml	10 ml	
8	Wash Buffer(25X)	15ml	30 ml	
9	Plate Sealer	3 pieces	5 pieces	
10	Product Description	1 copy	1 copy	

Additional materials required:

1. Microplate reader (wavelength: 450nm)

2. 37°C incubator (CO2 incubator for cell culture is not recommenced.)

3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)

4. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips (calibration is required before use.)

5. Sterile tubes and Eppendorf tubes with disposable tips

6. Absorbent paper and loading slot

7. Deionized or distilled water

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plate is kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

10. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.

11. Please wear the lab coat, mask, and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples. Please follow regulations on safety protection of biological laboratory.

5. Sample Preparation

Serum, Plasma, Cell culture supernatant, Cell or tissue lysate, Other liquid samples

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolysed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1000 x g. Collect the clear supernatant and assay immediately.

Cell lysates:

Suspension Cell Lysate:

1. Centrifuge at 2500 rpm at 2-8℃ for 5 minutes, then collect the cell.
2. Add pre-cooling PBS into collected cell and gently mix; Centrifuge at 2500 rpm at 2 -8℃ for 5 minutes, then collect the cell.
3. Add 0.5-1ml of RIPA lysis buffer (medium). The RIPA lysis buffer should have a pH of 7.3. It is not recommended to use components that contain NP-40 lysis buffer, Triton X-100 surfactant, or DTT with higher concentrations, as they may interfere with the antigen-antibody reaction. Alternatively, you can use a solution of 50mM Tris, 0.9% NaCl, and 0.1% SDS at pH 7.3. Add some protease inhibitors, such as PMSF, at a working concentration of 1mmol/L. To lyse the cells, keep them on ice for 30 minutes to 1 hour. During the lysate process, either use a pipette tip for pipetting or intermittently shake the centrifugal tube to

ensure complete protein lysis. If sticky DNA appears, ultrasound can be used to disrupt it. (Another option for processing the sample on ice is to use an ultrasonic condition with a 3-5mm probe, 150- 300W power, 3-5 seconds per cycle, and 30 seconds intervals for 1-2 seconds of working time.)

4. After lysate or ultrasonic disruption, centrifuge the mixture at 10000rpm at 2-8℃ for 10 minutes. Then, transfer the supernatant into an EP tube. The assay should be performed immediately, or the sample can be stored at -20 or -80℃.

Adherent Cell Lysate:

1. Remove the supernatant and wash three times with pre-cooled PBS.
2. Add 0.5-1ml RIPA lysis buffer and some protease inhibitors (read requirements in suspension cell). Scrape adherent cell gently with a cell scraper.
3. Add the cell suspension to a centrifuge tube. Lyse the cells on ice for 30 minutes to 1 hour. Alternatively, disrupt the cells using ultrasound (refer to the requirements for suspension cells).
4. After lysate/ultrasonic disruption, centrifuge the mixture at 10,000rpm for 10 minutes. Then, transfer the supernatant into an EP tube. The assay should be performed immediately, or the sample can be stored at -20 or -80℃.

Notes: It is recommended to use ultrasound to disrupt cells during cell lysate preparation. Ultrasound can efficiently break DNA. DNA fragments will not significantly interfere with the performance of the ELISA kit.

Tissue Homogenates:

Place the target tissue on ice and wash it with pre-chilled PBS buffer (0.01 M, pH 7.4) to remove residual blood. Weigh the tissue for further use. Grind the tissue in lysate on ice. The volume of lysate to add depends on the tissue weight. Typically, 9 mL of PBS is used per gram of tissue. It is recommended to add protease inhibitors (e.g., 1 mM PMSF) to the PBS. Homogenize further using ultrasonic disruption or freeze-thaw cycles. (During ultrasonic disruption, use an ice bath for cooling. Freeze-thaw cycles should be repeated twice.) Centrifuge the homogenates at 5000×g for 5 minutes. Collect the supernatant for immediate analysis, or aliquot and store at -20°C or -80°C for future assays.

Measure the total protein concentration using a BCA assay kit for further analysis. For ELISA assays, the protein concentration should typically be between 1-3 mg/mL.

Notes: Tissue samples like liver, kidney, or pancreas, which contain higher endogenous peroxidase activity, may cause false positives by reacting with the TMB substrate. In such

cases, treat the samples with 1% H_2O_2 for 15 minutes to inactivate the peroxidase before proceeding with the assay.

PBS buffer or mild RIPA lysis buffer can be used for cell lysis. When using RIPA lysis buffer, ensure the pH is adjusted to 7.3. Avoid using reagents containing NP-40, Triton X-100, or DTT, as these can severely inhibit the performance of the kits. We recommend using a lysis buffer composed of 50 mM Tris, 0.9% NaCl, and 0.1% SDS, pH 7.3.

Other Biological Sample: Centrifuge samples for 15 minutes at 1000×g at 2-8℃. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

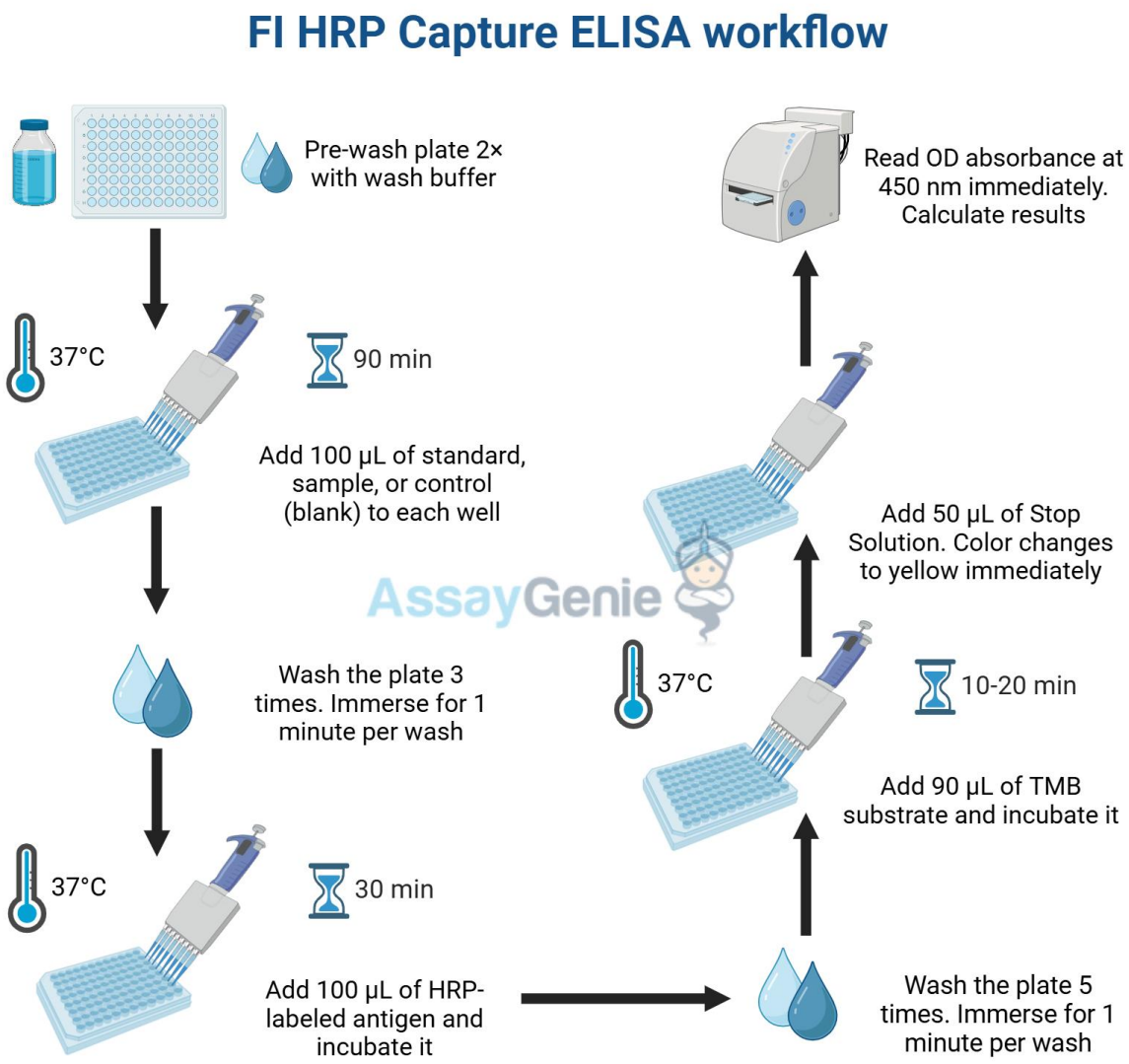
General Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Haemolysed samples are not suitable for this assay.

Notes for Samples

1. Blood Collection Tubes: Use disposable, non-endotoxin blood collection tubes. Avoid using hemolyzed or lipemic samples.
2. Optimal Sample Storage Conditions: Store samples at 2-8°C for up to 5 days. Store at -20°C for up to 6 months. Store at -80°C for up to 2 years. For long-term storage, samples can be stored in liquid nitrogen. When thawing frozen samples, use a rapid water bath (15-25°C) to minimize the formation of ice crystals at 0°C. After thawing, centrifuge the samples to remove any precipitate, then mix well.
3. Detection Range: The detection range of this kit does not directly correspond to the analyte concentration in the sample. For samples with higher or lower concentrations, dilution or concentration may be necessary.
4. Pretest Recommendation: For special samples with no reference data, a pretest is recommended to validate the assay's accuracy and reliability.
5. Recombinant Protein Compatibility**:** Recombinant proteins may not bind effectively to the capture or detection antibodies in the kit, which could result in undetectable assay results.

6. Workflow Overview



7. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and select the optimal dilution factor to ensure the protein concentration falls within the kit's recommended detection range. Dilute the samples using the dilution buffer provided with the kit. Multiple dilution tests may be necessary to achieve optimal results. Ensure that the test samples are thoroughly mixed with the dilution buffer. Both standard and sample dilutions should be prepared prior to starting the experiment.

Note: Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

Dilute 30 mL of concentrated wash buffer (15 mL for 48T) with deionized or distilled water to a final volume of 750 mL (375 mL for 48T) and mix well. The recommended resistivity of ultrapure water is 18 MΩ·cm. Alternatively, prepare a 25-fold dilution by adding the appropriate amount of concentrated wash buffer, then mix well. Store any unused solution at 2-8°C.

Crystals that form in the concentrated wash buffer can be dissolved by placing the solution in a water bath at 40°C until fully dissolved (do not exceed 50°C). Mix well before proceeding to the next step. It is recommended to use the prepared wash buffer within one day. Any remaining buffer can be stored at 2-8°C for up to 48 hours.

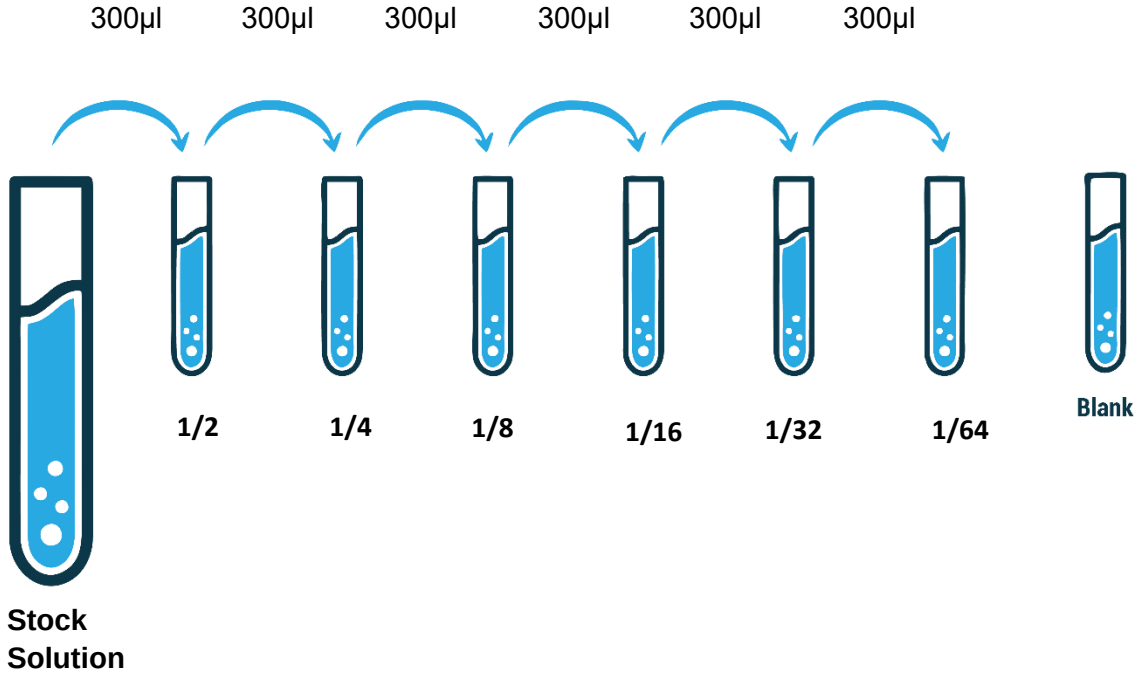
2. Standard Dilution:

1) Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 10 min and mix thoroughly.

Note: If the standard vial concentration is different to the highest value in the range (please see page 3), please dilute using sample buffer to match highest range value to create stock solution.

2) Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 300µl of the Sample dilution buffer into each tube. Add 300µl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 300µl from 1st tube to 2nd tube and mix thoroughly. Transfer 300µl from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample dilution buffer is used as blank control.

DILUTION SERIES



Note: Standard solutions are best used within 2 hours of preparation. The standard solution series can be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of of HRP-labeled Antigen Working Solution

The working solution should be prepared no more than 30 minutes before the assay and should not be stored for extended periods.

1. Calculate the required total volume of the working solution: Multiply 100 µL per well by the number of wells to be used. It is recommended to prepare an additional 100–200 µL to account for any losses.

2. Centrifuge: Spin the solution at 1000×g for 1 minute at low speed to bring the concentrated biotin-labeled antibody to the bottom of the tube.

3. Prepare the HRP detection antigen: Dilute the HRP-labeled antigen with the antigen dilution buffer at a 1:99 ratio and mix thoroughly. For example, add 10 µL of concentrated HRP-labeled antigen to 990 µL of antigen dilution buffer.

7. Assay Procedure

Before adding to the wells, equilibrate the TMB substrate for at least 30 mins at 37°C. When diluting samples and reagents, ensure they are mixed completely and evenly. It is recommended to plot a standard curve for each test.

Assay Procedure Summary

Step 1: Wash plate 2 times before adding Standard, Sample and Control (blank) wells.

Step 2: Add 100ul standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1min each time.

Step 3: Add 100ul HRP-labeled antigen working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 5: Add 50ul 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. Wash plate 2 times before adding Standard, Sample and Control (blank) wells.

2. **Standards and samples loading**: Aliquot 100ul of blank, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube into each standard well. Also add 100ul sample dilution buffer into the control (blank) well. Then, add 100ul pilot samples into each sample well. Seal the plate and static incubate for 90 minutes at 37°C. (Add the solution to the bottom of each well. Mix gently and without touch the sidewall and foam the sample.)

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 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.

4. **HRP-labeled Antigen**: Add 100ul HRP-labeled antigen working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C.

5. **Wash five times:** Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 3.

6. **TMB Substrate:** Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.

(Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can stop the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable.

7. **Stop:** Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow 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 and calculate.

8. Data Analysis

Calculate the mean O.D.450 value of the duplicate reading of each standard, control and sample.

Then calculate the relative O.D.450 using the following equation:

The relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well)

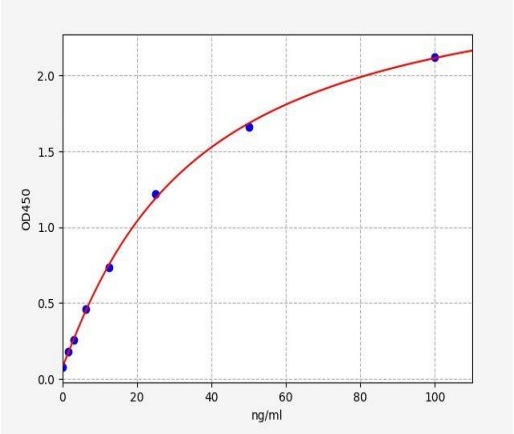
The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). (Do not plot the blank). The concentration of the samples can be determined from the standard curve. It is recommended to use professional software such as curve expert 1.3 or 1.4. to create a four parameter logistic curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

9. Typical Data & Standard Curve

Standard Curve

Results of a typical standard run of this ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. **Each user should obtain their own standard curve as per experiment.**



Specificity

This assay has high sensitivity and excellent specificity for detection of Rabbit OVA sIgE . No significant cross-reactivity or interference between Rabbit OVA sIgE and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between Rabbit OVA sIgE and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with a certain level of Rabbit OVA sIgE and the recovery rates were calculated by comparing the measured value to the expected amount of Rabbit OVA sIgE in the samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	94-103	99
EDTA Plasma(n=5)	85-105	96
Heparin Plasma(n=5)	85-101	94

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Rabbit OVA sIgE and their serial dilutions.

Sample	1:2	1:4	1:8
Serum(n=5)	85-104%	86-99%	86-96%
EDTA Plasma(n=5)	90-101%	86-101%	85-100%
Heparin Plasma(n=5)	88-100%	85-100%	87-99%

Precision

Intra-assay Precision: samples with low, medium and high concentration were tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration were tested 20 times on three different plates.

Stability

The stability of the Rabbit OVA sIgE ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

Standard (n=5)	37°C for 1 month	4°C for 6 months
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end**.**

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