Technical Manual

HRP-SA (Streptavidin Horseradish Peroxidase) Activity ELISA Kit

- Catalogue Code: AEFI01231
- Direct ELISA Kit
- Research Use Only

Contents

1. Key features and Sample Types

Aliases:

HRP-SA ELISA Kit, Streptavidin Horseradish Peroxidase ELISA Kit

Detection method:

Direct ELISA

Sample Type:

Serum, Plasma & Other Biological Samples

Reactivity:

Streptavidin-horseradish peroxidase

Range:

28.125 - 1800uU/ml (1800uU=6ng)

Sensitivity:

14uU/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 Direct ELISA kit allows for the quantitative measurement of HRP-SA (Streptavidin Horseradish Peroxidase) Activity in the following samples: serum, plasma and other biological samples.

How do our ELISA kits work?

This kit is based on Direct ELISA technology. Biotin is pre-coated onto 96-well plates. The general antibodies present in the sample are captured. The standards, test samples were added to the wells subsequently, and washed with wash buffer. Unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated. 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	Liquid Standard (1800 uU/ml)	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	TMB Substrate	5ml	10 ml	2-8°C (Avoid Direct Light)
4	Sample Dilution Buffer	10ml	20 ml	2-8°C
5	Stop Solution	5ml	10 ml	
6	Wash Buffer(25X)	15ml	30 ml	
7	Plate Sealer	3 pieces	5 pieces	
8	Product Description	1 copy	1 copy	

Additional materials required:

1. Microplate reader (wavelength: 450nm)

2. 37°C incubator (CO_2 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 all the contents.

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

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.

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

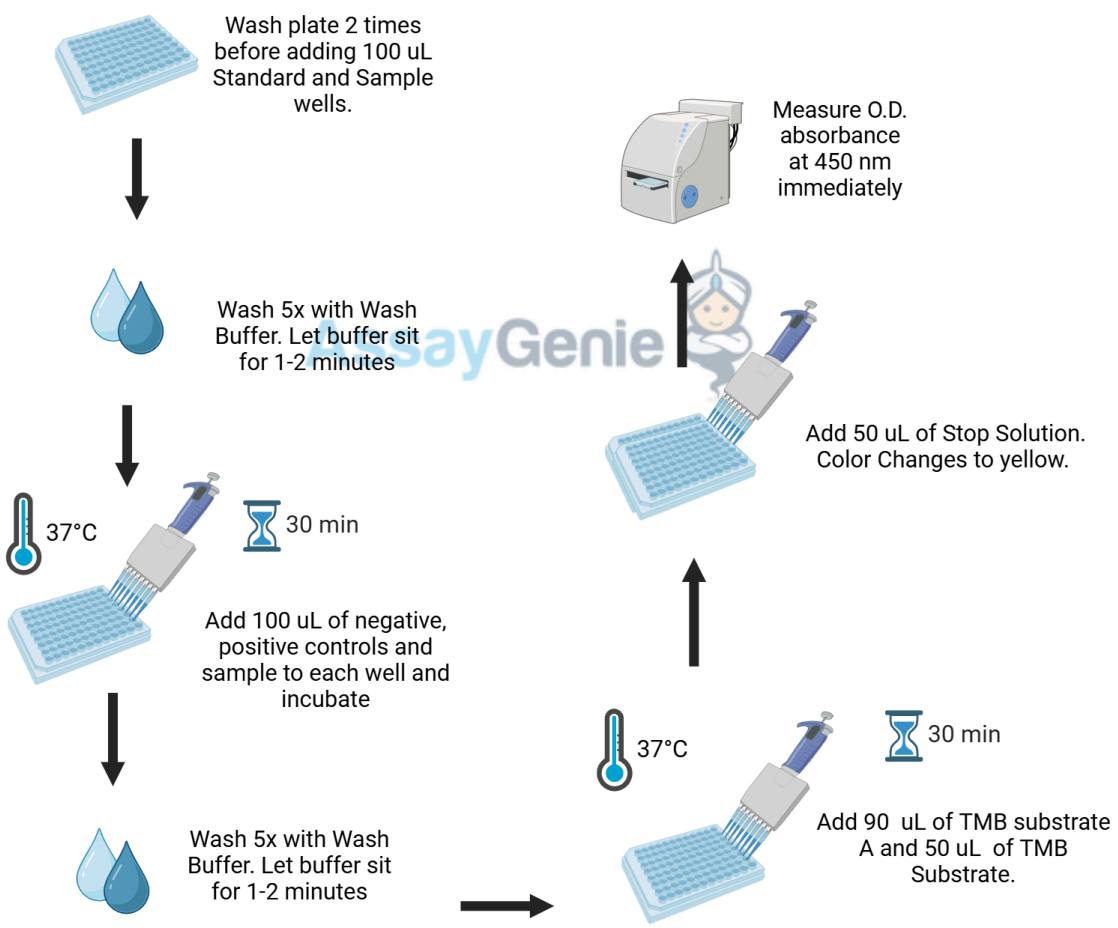
1.Optimal Sample Storage Conditions: Store samples at 2-8°C for up to 5 days. Store at -20°C for up to 6 months. Store at -80°C for up to 2 years. For long-term storage, samples can be stored in liquid nitrogen. When thawing frozen samples, use a rapid water bath (15-25°C) to minimize the formation of ice crystals at 0°C. After thawing, centrifuge the samples to remove any precipitate, then mix well.

2.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.

3.Pretest Recommendation: For special samples with no reference data, a pretest is recommended to validate the assay's accuracy and reliability.

4.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.

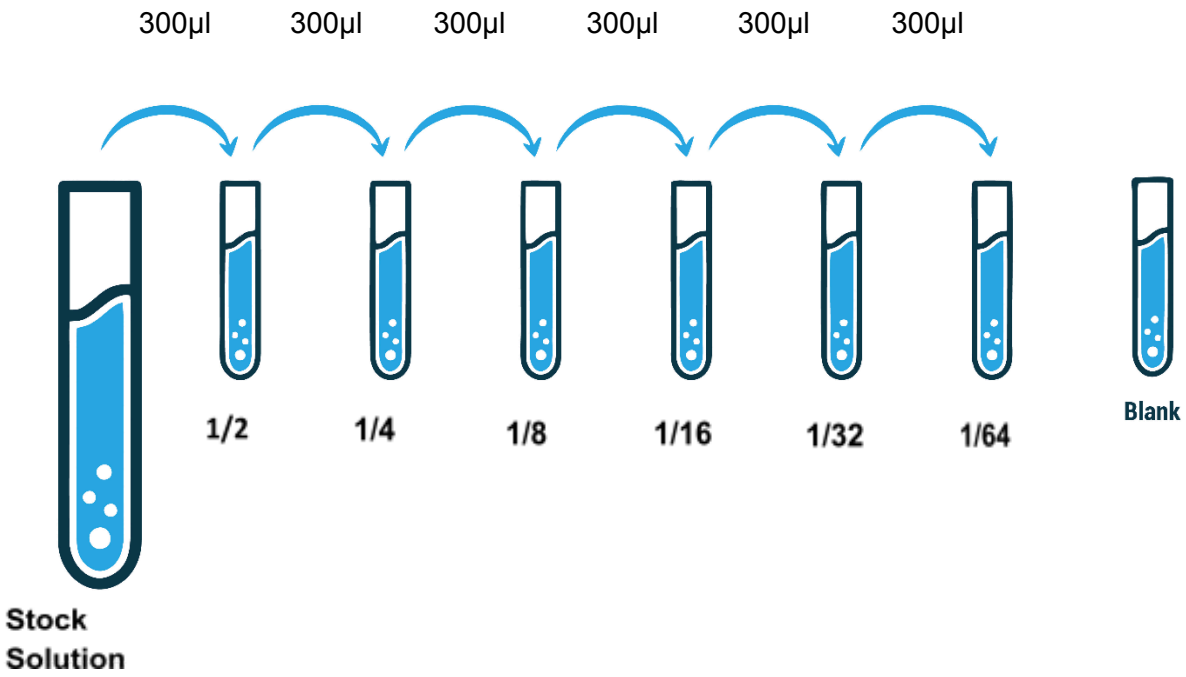
2. Standard Dilution:

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.

8. 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 and Sample wells.

Step 2: Add 100ul standard or sample to each well and incubate for 30 minutes at 37°C. Wash step: Aspirate and wash plates 5 times.

Step 3: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step 4: Add 50ul Stop Solution. Read at 450nm immediately and calculation.

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. **Prepare Standards & Sample loading:** Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube and Sample Dilution Buffer (blank) into the standard wells. Add 100ul of properly diluted sample into test sample wells.

3. **Wash five times:** Wash with wash buffer 5 times. Let the buffer sit for 1-2 minutes.

4. **Incubate:** Seal the plate with a cover and incubate at 37°C for 30 minutes.

5. **Wash five times:** Remove the cover, and then wash the plate with wash buffer five times. Read the 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: 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 gradient appeared in standard wells.)

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.

(Notes: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution)

9. 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.

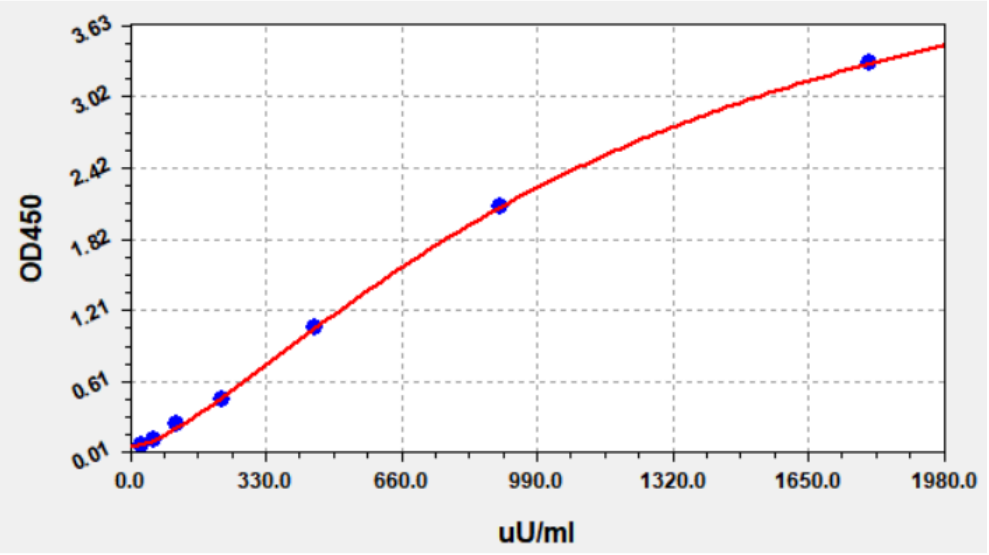
Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

10. 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.**

STD (uU/ml)	OD-1	OD-2	Average	Corrected
0.0	0.05	0.05	0.05	0.0
28.125	0.075	0.071	0.073	0.023
56.25	0.112	0.108	0.11	0.06
112.5	0.252	0.248	0.25	0.2
225.0	0.466	0.456	0.461	0.411
450.0	1.072	1.069	1.07	1.02
900.0	2.101	2.078	2.089	2.039
1800.0	3.317	3.289	3.303	3.253



Precision

- Intra-assay Precision: CV < 8%
- Inter-assay Precision: CV < 10%

Stability

The stability of 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 condition.

Standard (n=5)	37°C for 1 month	2-8°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.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

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