FineTest®

HRP-SA (Streptavidin Horseradish Peroxidase) Activity ELISA Kit

Catalogue No.: EU2619

Revision: V 1.0

Size: 48T/96T

Reactivity: Streptavidin-horseradish peroxidase

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

Sensitivity: 14uU/ml

Application: For quantitative detection of HRP-SA (Horseradish peroxidase conjugated Streptavidin).

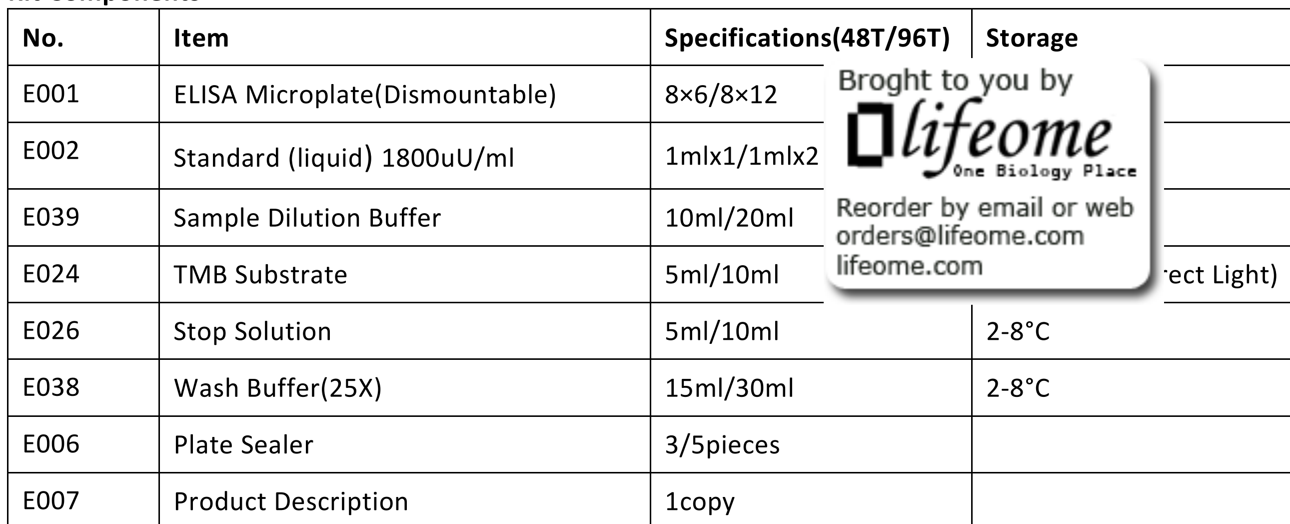
Storage: 2-8°C

Expiry Date: see kit label

Principle: Direct

NOTE: FOR RESEARCH USE ONLY.

Kit Components



No.	Item	Specifications(48T/96T)	Storage
E001	ELISA Microplate(Dismountable)	8×6/8×12	[illegible]
E002	Standard (liquid) 1800uU/ml	1mlx1/1mlx2	[illegible]
E039	Sample Dilution Buffer	10ml/20ml	[illegible]
E024	TMB Substrate	5ml/10ml	ect Light)
E026	Stop Solution	5ml/10ml	2-8°C
E038	Wash Buffer(25X)	15ml/30ml	2-8°C
E006	Plate Sealer	3/5pieces	
E007	Product Description	1copy	

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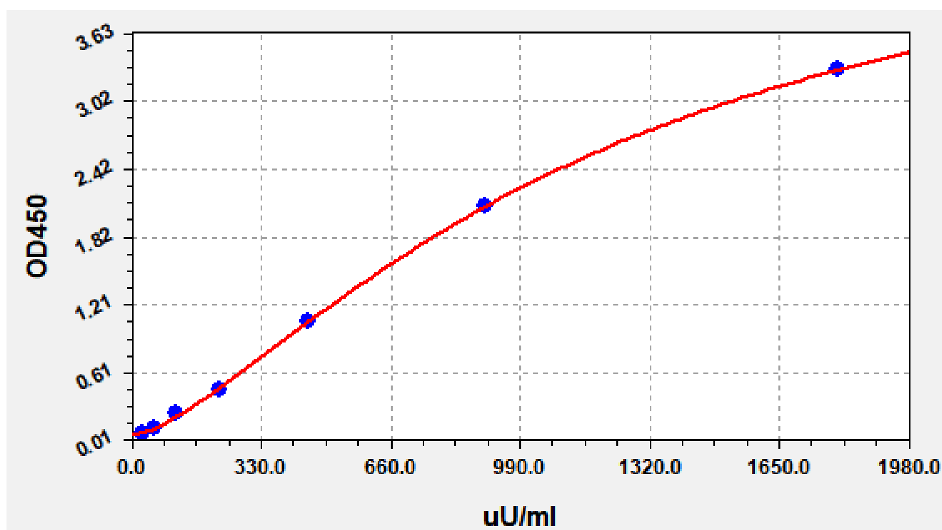
Typical Data & Standard Curve

Results of a typical standard operation of a HRP-SA activity ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves.

STD.(uU/ml)	OD-1	OD-2	Average	Corrected
0	0.05	0.05	0.05	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	0.250	0.200
225	0.466	0.456	0.461	0.411
450	1.072	1.069	1.07	1.02
900	2.101	2.078	2.089	2.039
1800	3.317	3.289	3.303	3.253



Precision

Intra-Assay: CV<8%

Inter-Assay: 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 performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

Operation Procedure

Elisa Kits Operation Guide & Targeted control of TMB coloring

www.fn-test.com/category/knowledge-share/

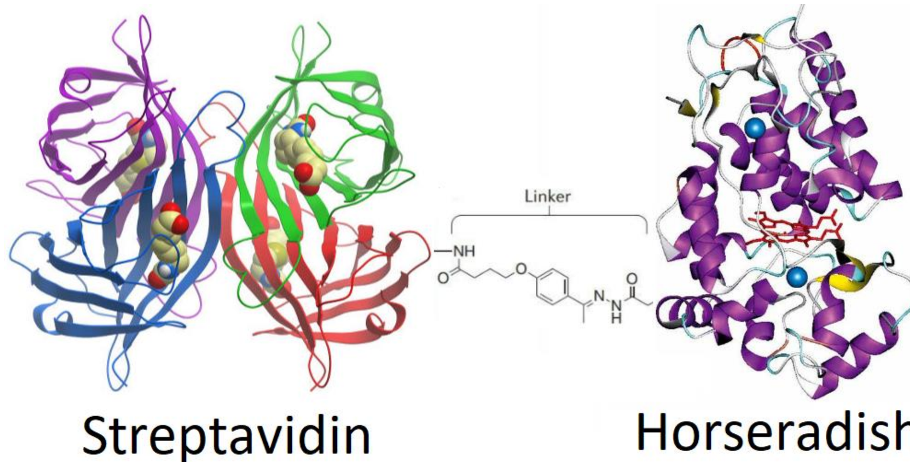
Principle of the Assay

This kit was based on direct enzyme-linked immune-sorbent assay technology. Biotin was pre-coated onto 96-well plates. 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.

Streptavidin: Recombinant, expressed in E. coli, a tetrameric protein with a globular structure, 18 units/mg (using green) 53.2KD.

Horseradish peroxidase: Natural, 300 units/mg (using pyrogallol), 44KD

Streptavidin-Horseradish peroxidase: The coupling ratio is 1:1.



Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

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

Note: Samples used within 5 days can be stored at 2-8°C; otherwise, they must be stored at -20°C or -80°C or liquid nitrogen to avoid loss of biological activity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this test.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation and Storage

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

1, Wash Buffer:

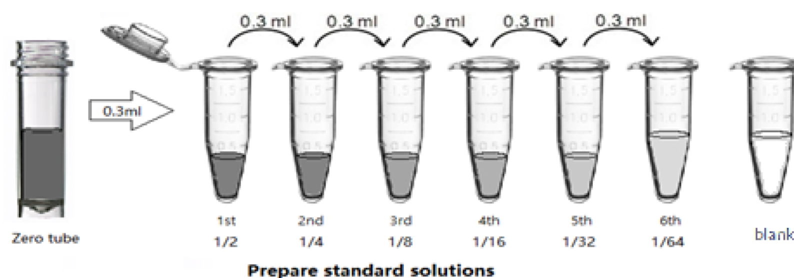
If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Dilute 30ml (15ml for 48T) Concentrated Wash Buffer to 750ml (375ml for 48T) Wash Buffer with deionized or distilled water (The recommended resistivity of deionized or distilled water is 18MΩ). Put unused solution back at 2-8°C.

2, Standards:

1). Label Standard tube as zero tube.

2). Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Prepare standard solutions

Note: It is best to use Standard Solutions within 1 hour.

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate. **Wash plate 2 times before adding standard, sample and control (blank) wells!**
2. **Prepare Standards:** Aliquot 100ul of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.
3. **Add Samples:** Add 100ul of properly diluted sample into test sample wells.
4. **Incubate:** Seal the plate with a cover and incubate at 37°C for 30 minutes.
5. **Wash:** Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
6. **TMB Substrate:** Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. **(Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C.).** (**Note:** 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:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
8. **OD Measurement:** Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank 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). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as **Curve Expert 1.3 or 1.4.**

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

Summary

Step 1: Wash plate 2 times before adding Standard and Sample wells!

Step2: Add 100ul standard or sample to each well and incubate for 30 minutes at 37°C.

Wash step: Aspirate and wash plates 5 times.

Step3: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step4: Add 50ul Stop Solution. Read at 450nm immediately and calculation.