Vero HCP ELISA Kit

Catalog No: E-FS-E146

96T

Version Number:	V1.1
Replace version:	V1.0
Revision Date:	2026.02.02

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Test principle

This kit is based on the solid-phase enzyme-linked immunosorbent assay and uses the double-antibody sandwich method to detect the residual amount of Vero expressed bacterium HCPs in the sample. This analytical method captures the HCPs in the sample by coating a polyclonal antibody against Vero HCPs on the microplate. At the same time, adds calibrators or test samples to a 96-well plate, and combines with HRP-labeled anti-Vero HCPs antibody. After incubation, they were washed; TMB was added for reaction; HRP catalyzes the oxidation of TMB by H_2O_2 to generate a blue product , and then the termination solution was added to terminate the enzymatic reaction, generating a yellow product. The absorbance value was measured at 450 nm using an enzyme analyzer. The absorbance was positively correlated with the HCPs concentration in the calibrator and sample.

Technical indicator

Reaction mode (Incubation time and temperature)**:** 25℃, 3h, 10min.

Detection limit: 3 ng/mL.

Cross-reactivity: Sf9, E.coli, Pichia Pastoris cell protein --- < 0.1%.

Kits components

Item	Specifications
ELISA Microtiter plate	96 wells
Vero HCP Standard	2 vials
Standard Diluent	1.5 mL
Diluent	25 mL * 2
100×Concentrated HRP Conjugate	120 μL
10×Concentrated Wash Buffer	25 mL * 2
Substrate Reagent	12 mL
Stop Solution	6 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instrument: Microplate reader, Printer, Homogenizer, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensibility: 0.01g).

High-precision transferpettor: Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid th e kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E146. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E146 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.8 unit (A450nm < 0.8), it indicates the reagent be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit descriptio-n. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8℃. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8℃. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. **Sample pretreatment Notice:**

   Experimental apparatus should be clean and the pipette should be disposable to avoid cross-contamination during the experiment.

2. **Solution preparation**

   Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

   1) Prepare **Vero HCP Standard Solution**: Refer to the vial label for reconstitution volume. Dissolve **Vero HCP Standard** with 500 μL of **Standard Diluent**, gently mix and invert, then let it stand for 5 minutes to obtain the reconstituted standard solution.

   Note: The calibration standard cannot be dissolved using solutions of other volumes. Calculate the concentration of the reconstituted standard based on the label infomation, and then perform gradient dilution.

   2) Prepare **Vero HCP Standard Curve Solution:** Refer to Table 1 to perform stepwise dilution of the standards**.**

   Table 1：

Standard Curve	**Prepare**	**Concentration** (ng/mL)
ST1	Dilute **Vero HCP Standard Solution** to the ST1 concentration using the **Diluent**.	243
ST2	300 μL ST1 + 600 μL Diluent	81
ST3	300 μL ST2 + 600 μL Diluent	27
ST4	300 μL ST3 + 600 μL Diluent	9
ST5	300 μL ST4 + 600 μL Diluent	3
ST6	300 μL ST5 + 600 μL Diluent	1
NCS (0 ng/mL Standard)	Diluent	0

   3) **HRP Conjugate Solution:** Dilute **100×Concentrated HRP Conjugate** with **Diluent**. (100×Concentrated HRP Conjugate (V): Diluent (V) = 1:99)

   4) **Wash Buffer:** Dilute the **10×Concentrated Wash Buffer** with deionized water. (10×Concentrated Wash Buffer (V): Deionized water (V) =1:9)

3. Sample pretreatment procedure

- **Sample:** Samples from the expression and purification process, including stock solutions, etc. They should be clear and transparent, and can be made clear by means such as centrifugation or filtration to remove insoluble substances.
- **Storage:** The samples must undergo stability studies in advance to determine the optimal storage conditions; generally, it is recommended that the samples be stored at temperatures below -65 ℃ for a long time, and they should not be repeatedly frozen and thawed.
- **Processing:** Dilute the test sample according to its estimated HCPs concentration by an appropriate factor, ensuring the measured value falls within the calibrated curve's valid range.
- For initial testing or when sample HCPs levels are unknown, strongly recommend performing a preliminary suitability verification using appropriately diluted samples to better inform subsequent routine testing.

Note: Validation protocols are available upon request from our technical support team.

Assay procedure

Restore all reagents and samples to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard (**ST1-ST6, NCS**) and Samples need test in duplicate.**
2. **HRP Conjugate:** add 100 μL of **HRP Conjugate Solution** to each well.
3. **Add sample:** then add 100 μL of **Standard** or **Sample** per well, cover the plate sealer and place it on a microplate shaker. Incubate at 25℃ at 600 rpm for 3 h in shading light.
4. **Wash:** uncover the sealer carefully, remove the liquid of each well. Immediately add 350 μL of **Wash Buffer** to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean with absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **Color Development:** add 100 μL **Substrate Reagent**, gently oscillate for 10 s to mix thoroughly. Incubate at 25℃ for 10 min in shading light. (The reaction time can be extended according to the actual color change).
6. **Stop reaction:** add 50 μL of **Stop Solution** to each well. Gently oscillate to mix thoroughly. Read the value immediately after Stop reaction.
7. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader.

Result analysis

1. **Absorbance = A-A0**

   A: Average absorbance of standard or sample

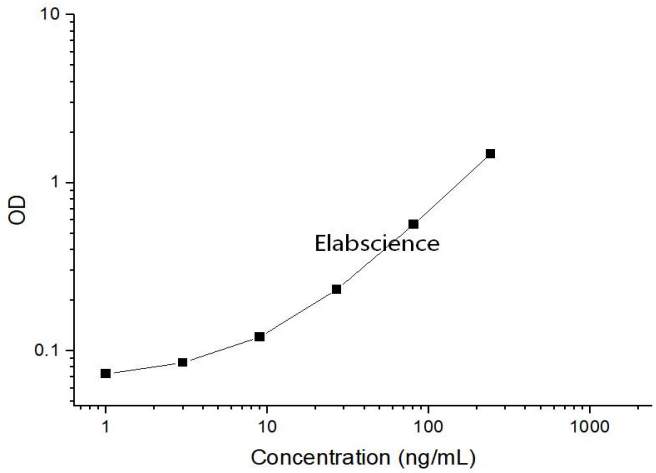
   A_0: Average absorbance of 0 ng/mL Standard

2. **Drawing and calculation of standard curve:**

   The standard curve equation is obtained by performing a four-parameter logistic fit using the standard concentration and OD values. The average OD value of the sample is substituted into the equation to calculate the sample concentration, which must then be multiplied by the dilution factor to obtain the actual concentration of the sample.

   Curve-fitting software built into microplate readers can be used for this calculation. If unavailable, professional curve-fitting software such as Curve Expert or ELISA Calc should be adopted.

Vero HCP (E-FS-E146) **Standard Curve**



Limitations of test method

1. This product is intended for research use only and is not for clinical diagnosis.
2. This product is only applicable for the detection of protein content in host cells derived from the Vero expression strain. For other types of expression strains, suitability verification is required.
3. Sample pH should be maintained within the range of 6.5–8.5; pH values outside this range may lead to abnormal measurement results.

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