Instructions for Use of Sf9 HCP ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- HCP004

Introduction

BlueKit series Sf9 HCP ELISA Detection Kit is a specialized kit for the quantitative detection of residual insect cell Sf9-derived host proteins.

The polyclonal antibody in this kit is prepared by using as antigen cleaving HCPs obtained from sf9 cells followed by immunizing sheep to obtain serum and obtaining high-quality antibody by affinity purification method. A double-antibody sandwich method is used for co-incubation by adding calibrators or samples to be tested and HRP-labeled anti-Sf9 HCPs polyclonal antibodies to microtiter plates precoated with anti-Sf9 HCPs polyclonal antibodies; After washing, the color reaction is developed using the added TMB substrate, and finally the enzyme-catalyzed reaction is terminated with stop solution. Absorbance values are measured and read by a microplate reader at 450 nm, which are positively correlated with the concentration of HCPs in the calibrator and sample, and the concentration of Sf9 HCPs in the sample can be calculated from the dose-response curve.

Assay range: 3-243 ng/mL

Limit of quantification: 3 ng/mL

Specification

96 T

Usage

It's applicable to biological products based on insect cell-baculovirus expression system, including but not limited to recombinant proteins, vaccines, gene therapy AAV vectors, etc.

Kit components

Components	Specification	Preparation
Sf9 HCP Standard	Lyophilized powder × 3 vials	Dissolve with 500 µL of reconstitution solution and allow to stand for approximately 5 min.The solution should be clear and transparent with no visible insoluble matter.
Coated Plate	8 wells × 12 strips	Ready-to-use
Standard Reconstitution Solution	1.5 mL × 2 tubes	Ready-to-use
Dilution Buffer	25 mL x 2 vial	Ready-to-use
Concentrated Buffer (10×)	25 mL × 2 vials	Make a 10-fold dilution with ultrapure water.
Sf9 HCP Enzyme-labeled Antibody (100×)	120 µL × 1 tube	Make a 100-fold dilution with diluent.
TMB Substrate	12 mL × 1 vial	Ready-to-use
Stop Solution	6 mL × 1 vial	Ready-to-use
Sealing Film	5 pieces	Ready-to-use
Instructions for Use	1 copy	Ready-to-use

Storage and shelf life

Sealed kits are valid for 12 months at 2-8℃.

Storage requirements for unsealed components are as follows:

Microtiter plate: The unsealed microtiter plate strip shall be sealed and stored in a zip lock bag together with desiccant, and it can be stable for 30 days at 2-8℃ after validation.

Reconstitution of calibrator: The calibrator can be stored at 2-8℃ on the day of dissolution. After use, transfer to ≤ -18℃ for storage on the day of use. Repeated freezing and thawing should not exceed 3 times.

Apparatus and materials to be prepared by the user:

- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized water
- Unused filter paper
- Vortex shaker

Pre-experiment preparation

1. Take out the coated plate and equilibrate at room temperature for approximately 20 min. All other reagents shall be taken out in advance before use and equilibrated at room temperature; Return to 2-8℃ for storage immediately after use.

2. Calculate the number of wells required according to the number of test samples, take out the corresponding number of pre-coated ELISA plate strips, seal the remaining strips with desiccant in a zip lock bag, put them back into the kit and store in a refrigerator at 2-8℃, and use the kit up within the shelf life.

3. Dissolution of Sf9 HCP calibrator: Accurately measure 500 µL of the calibrator reconstitution solution into an upright cryogenic vial, mix by gentle inversion, and allow to stand for 5 min. The HCP concentration of the reconstituted calibrator is the nominal concentration. The dissolved calibrator should be stored at ≤ -18℃, and repeated freezing and thawing shall not exceed 3 times.

4. Preparation of 1× buffer: Dilute the concentrated buffer (10×) by 10 times with ultrapure water. For example, take 25 mL concentrated buffer (10×) and add 225 mL ultrapure water to mix well to obtain 1× buffer, which is used for washing the plate. It's recommended to prepare for immediate use. If a plate washer is used for washing, the reagent may be insufficient and buffers of the same product number may be purchased separately.

5. Preparation of detection antibody: Dilute it by 100-fold with diluent in a sterile centrifuge tube, gently invert to mix well and obtain 1× anti-Sf9 HCP enzyme-labeled antibody. Prepare a suitable volume to ensure sufficient margin when adding the liquid. It shall be newly prepared for immediate use.

6. Preparation of calibration curve: Perform gradient dilution for calibrators according to the following table.

Calibration Curve Sample	Loading	Concentration (ng/mL)
ST1	Dilute the stock calibrator solution to STI concentration with diluent	243
ST2	300µL ST1 + 600µL Dilution Buffer	81
ST3	300µL ST2 + 600µL Dilution Buffer	27
ST4	300µL ST3 + 600µL Dilution Buffer	9
ST5	300µL ST4 + 600µL Dilution Buffer	3
ST6	300µL ST5 + 600µL Dilution Buffer	1
NCS	Dilution Buffer	0

Remark: 1 ng/mL is used as an anchor point to fit the calibration curve, but its concentration CV and relative deviation are not required in the methodology validation.

Operation procedures

1. Adding detection antibody: Add 1 × Sf9 HCP enzyme-labeled antibody solution into the reagent reservoir, and quickly add

antibody solution into the bottom of microplate well by 100 μL/well with a multi-channel pipette, without introducing bubbles. In the actual test, samples can be loaded according to the number of samples (96-well plate can be arranged with reference to the following table).

	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS	NCS	NCS		S1	S1	S1					
B					S2	S2	S2					
C	ST6	ST6	ST6		S3	S3	S3					
D	ST5	ST5	ST5		S1+SRC	S1+SRC	S1+SRC					
E	ST4	ST4	ST4		S2+SRC	S2+SRC	S2+SRC					
F	ST3	ST3	ST3		S3+SRC	S3+SRC	S3+SRC					
G	ST2	ST2	ST2									
H	ST1	ST1	ST1									

Remark: The illustration represents the calibration curves of 6 concentration gradients detected (STD1-STD6), 1 NTS, 3 samples to be tested (S1-S3) and spike recovery SRC of each sample (S1 SRC-S3 SRC). In the actual test, the 96-well plate can be arranged and samples are loaded according to the sample size.

2. Adding the calibrator and the sample to be tested: Accurately pipette 100 μL of the series of standard solutions, the diluent (0 value), and the sample to be tested into the corresponding microplate. Avoid any bubbles during operation.

3. After adding samples, seal the microplate with sealing film, place on the microplate constant temperature oscillator, protect from light and incubate for 3 hours at 600 rpm at room temperature.

4. Equilibrate the TMB chromogenic reagent at room temperature 20 min in advance.

5. Wash the above plate with 1× buffer by 300 μL/well, shake off the liquid quickly, and pat it dry on a paper towel, and repeat such washing for 5 times. After washing, the microplates should be immediately used for the following procedures and should not be put away.

6. Take appropriate volume of TMB chromogenic reagent into the reagent reservoir, quickly add TMB chromogenic reagent into the above microplate by 100 μL/well with a multi-channel pipette, and incubate at room temperature for 15 min in the dark. Do not seal with sealing film in this step.

7. Take appropriate volume of stop solution into the reagent reservoir, and quickly add stop solution into the above microplate by 50 μL/well with a multi-channel pipette.

Remark: The order of addition should be identical to that of adding chromogenic reagent. The tip should be suspended when adding the sample to avoid contact with the solution in the microplate, and do not produce any bubble. Read immediately after termination.

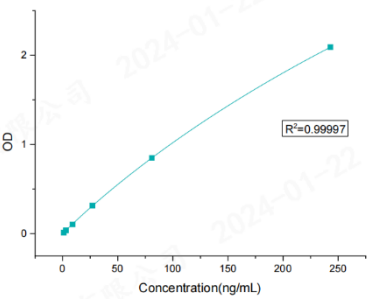
8. Set the detection wavelength as 450 nm and correction wavelength as 630 nm in the microplate reader, and measure the OD value of each well. Do not cover the sealing film or lid during testing.

Results processing

1. OD processing of the standard curve (See the following example, which is only for example purpose. Please refer to the actual measurement for details):

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
0.00	0.038	0.038	0.038
1	0.051	0.049	0.050
3	0.074	0.076	0.075
9	0.145	0.139	0.142
27	0.362	0.344	0.353
81	0.883	0.891	0.887
243	2.138	2.119	2.129

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.

2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.

3. Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4℃ for one month. Other unused reagents should be packaged or covered properly.

4. Please use disposable tips during experimental operation to avoid cross contamination.

5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.

6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.

7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.

8. The kit is intended for single use. Please use within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

Jiangsu Hillgene Biopharma Co., Ltd.
A. Building 4, Yuewang Intelligent Valley, No. 1463, Wuzhong Avenue, Wuzhong District, Suzhou, Jiangsu, China
T. +86-18013115357 / +86-4009001882