Principle

This product uses the ELISA double-antibody sandwich method to determine the content of *E. coli* host cell protein (HCP) in samples. Anti-*E. coli* host cell protein antibodies are coated on a solid-phase carrier micro-plate. Standards or test samples and *E. coli* HCP detection antibodies are added to form an antibody-antigen-detection antibody complex. After washing, a substrate solution (3,3',5,5'-tetramethylbenzidine, TMB) is added for color reaction. TMB is colorless initially and turns blue under the catalysis of HRP, and finally turns yellow under acidic conditions. The intensity of the yellow color is positively correlated with the amount of *E. coli* HCP in the sample.

Specification

32 T/ Kit

Usage

This kit is used for the quantification of host cell protein (HCP) in bio-products which expressed and amplified using Escherichia coli (*E. coli*).

This kit is only for research and is not suitable for medical diagnosis.

Product Performance

This kit has been fully evaluated, with a standard curve range of 2-100 ng/ml and a quantitation limit of 1.5 ng/mL.

The precision within the plate is less than 10%, and the precision between plates is less than 15%; the recovery rate is 80-120%.

This kit is only used for the detection of *E.coli* host cell protein (HCP) content in samples.

Main Components

Long-term storage at -20±5℃.Mix thoroughly before use and avoid repeated freeze-thaw cycles.

Components	Specification	Storage temperature
E.coli HCP Standard 1-8（Concentration：48, 24, 12, 4.8, 2.4, 1.2, 0.48, 0 ng）	8T/pkg×8	2-8℃
E.coli HCP antibody	4 mL×1 bottle	
Concentrated Wash Buffer（20×)	20 mL×1 bottle	
Sample Diluent	25 mL×1 bottle	
TMB Substrate	4 mL×1 bottle	
Stop Solution for TMB Substrate	3 mL×1 bottle	
Anti-HCP antibody ELISA plate	32 well plate	
Sealing film	1 sheet	
One Instruction Manual	1	

Items that need to be prepared additionally

1. Vortex Oscillator
2. Cleaner
3. Constant Temperature Micro-plate Shaker
4. Micro-plate Reader
5. Deionized water
6. Pipettor and tips
7. Absorbent paper,etc.

Storage conditions and shelf life

1.The shelf life of an unopened product is 12 months. After opening, the product is valid for 1 month (within the expiration date).
2.After the micro-plate is unsealed and used, the remaining portion should be stored sealed in an aluminum foil bag.
3.After use, it should be promptly returned and stored under 2-8℃ conditions.
4.The product batch number and expiration date can be found on the product packaging label.

Precautions

1. This kit is only used for research.
2. All samples and reagents are considered potentially hazardous. For your safety and health, please wear protective measures such as lab coats and disposable gloves. Avoid direct contact of reagents with skin and eyes. In case of accidental contact, rinse immediately with plenty of water.
3. Please read the product manual carefully before using this kit.
4. Please use the product within the expiration date indicated on the kit, and store each component according to the label conditions. Components from different batch numbers should not be mixed. This kit should not be used with other commercial kits.
5. To avoid microbial contamination, as well as cross-contamination between reagents and samples, please use disposable tips, use clean containers and sample wells to prepare and temporarily store reagents, and do not reuse sealing film.
6. All components should be brought to room temperature before use, and liquid reagents should be thoroughly mixed before sampling. The pre-made micro-plate is a detachable strip plate, and strips can be removed according to experimental needs. Remaining unused portions should be promptly returned to the foil bag containing a desiccant, sealed, and stored at a low temperature. Avoid scratching the bottom of the micro-plate or performing any other actions that could affect optical density measurements.
7. It is recommended to use a plate washer for washing plates. During the washing process, avoid cross-flow or bubbles to prevent affecting the accuracy of the experimental results. After the final wash, any remaining liquid in the wells should be emptied.
8. A new standard curve needs to be generated for each measurement.

Detection method

1.Preparation Before Experiment

Allow the required reagents to equilibrate at room temperature (25±2°C) for at least 30 minutes, until the reagents have returned to room temperature (25±2°C).

2.Reagent Preparation

If crystals appear in the concentrated reagent, place it in a 37°C water bath until all the crystals are completely dissolved.

2.1 Standard curve preparation

After the lyophilized 8-tube strips have equilibrated to room temperature (25±2°C), remove the outer packaging. Add 240 μL of sample diluent per well and mix gently by pipetting. Prepare for use immediately. Tube 1 contains the standard at a concentration of 200 ng/mL and is to be used for spike recovery. Tubes 2-8 are for the standard curve, with standard concentrations of 100, 50, 20, 10, 5, 2, and 0 ng/mL, respectively. Prepare immediately before use.

Notes: The standard must not be reconstituted with a different volume of reconstitution solution. Pay attention to the tube numbers; the standard curve tubes are numbers 2-8, do not confuse them. After reconstitution, the 8-tube strips can be secured by placing them into the pipette tip holes of a tip box.

2.2 Preparation of washing solution

After allowing the concentrated wash solution (20×) to reach room temperature (25±2°C), gently mix it. Calculate the required amount of wash solution according to the experiment. Dilute and prepare it at a volume ratio of concentrated wash solution (20×) to purified water = 1:19, gently mix to avoid excessive foaming. Prepare fresh as needed.

3.Sample dilution

It is recommended to conduct spike recovery studies on the samples to eliminate the interference of the sample matrix and ensure the accuracy and reliability of HCP detection results. It is recommended to dilute the samples using the minimum dilution factor.

If your sample is a process stage sample and its concentration is higher than the analytical range of this detection method, it can be diluted at a higher than minimum dilution factor, but the dilution linearity should be assessed to ensure that the detection method is accurate and has sufficient antibody excess to be suitable for your HCP.

4. Testing Steps

4.1 Micro-plate well arrangement

Take out the corresponding number of strips from the aluminum foil bag according to the experimental quantity.

4.2 Add detection antibody

Add 100μLof detection antibody to each well

4.3 Add standard/sample

Add 100μLof *E. coli* HCP standards of various concentrations, the prepared samples, and the negative control. Be sure to add the standards, samples, controls, and detection antibodies continuously within 15 minutes. Seal the plate with a plate seal, and incubate at room temperature (25±2°C) on a micro-plate shaker for 100 minute, keeping the shaker speed at 350 rpm (the speed can be adjusted according to different shakers to ensure the solution in each well is thoroughly mixed without splashing).

4.4 Washing

Discard the liquid in each well, then add 350μL of washing solution to each well. After standing for 30 seconds, discard the liquid in the wells; repeat this procedure 4 more times. After the final wash, dry the plate by patting it on blotting paper.

4.5 Add Substrate

Add 100μL of substrate solution to each well, keep at room temperature (25±2°C) and incubate in the dark for 30 minutes.

4.6 Stop the reaction

Add 50μLof stop solution to each well, mix gently, and observe the color change from blue to yellow.

4.7 Signal detection

Select the micro-plate reader's main wavelength at 450nm and the reference wavelength at 630nm to measure the absorbance values of each well at 450nm and 630nm. Please read the data within 15 minutes after the reaction is stopped.

5. Result Analysis

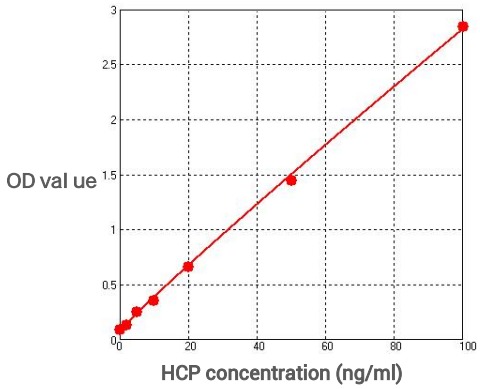
5.1 The OD value of each well = absorbance at 450nm - absorbance at 630nm. If the micro-plate reader is not equipped with a reference wavelength (630nm), the OD value of each well = absorbance at 450nm. If the OD value of the sample to be tested exceeds the maximum value of the standard curve, the sample should be further diluted before testing.

5.2 It is recommended to use the concentration of the standard as the x-axis and the OD value as the y-axis for this kit, and fit the curve using a four-parameter method. The standard curve fitting can be done using the software that comes with the micro-plate reader. If that is not available, it is recommended to use professional standard curve software, such as Curve Expert or ELISA Calc. The correlation coefficient R^2 of the standard curve should be ≥0.99; otherwise, the experiment is invalid.
5.3 It is recommended to set up a repeat hole, and the average value should be calculated before performing curve fitting.
5.4 Substitute the OD value of the sample to be tested into the fitted equation of the standard curve to calculate the sample concentration, which represents the concentration of *E. coli* host cell protein (HCP) in the sample. If the sample to be tested has been diluted, the calculated result should be multiplied by the dilution factor.

Typical Standard Curve

A typical standard curve is shown below; the data is for reference only and the actual test results shall prevail.

E. coli HCP Concentration ng /ml	OD value
100	2.846
50	1.442
20	0.659
10	0.356
5	0.249
2	0.135
0	0.088



Common Issue Troubleshooting

Abnormal phenomenon	Possible cause	Solution approach
The overall OD values of the experiment are relatively low	The kit is not fully balanced	Take the kit out of the refrigerator before the experiment and let it equilibrate at 25°C for at least 30 minutes before use.
	Improper mixing or storage of reagents	Reagents from different batches should not be mixed; please store each reagent according to the instructions in the manual.
	Incubation time/temperature does not match the instructions	Please strictly follow the instructions for incubation. In winter, when the room temperature is low, if the color development is lighter, you can appropriately extend the color development time or ensure the room temperature is adjusted to around 25°C.
	Improper sample preparation	Re-prepare standard curves and samples
The overall OD values of the experiment are higher than normal	Incubation time/temperature does not match the instructions	Please strictly follow the instructions in the manual for the incubation reaction.
	Improper sample preparation	Re-prepare standard curves and samples
Standard curve is normal, but the sample shows abnormal coloration	The sample dilution factor is not appropriate	It is recommended to make 4-5 dilution gradients for the initial test samples to ensure that at least one value falls within the detection range.
	Hook effect	Increase the dilution factor while ensuring the concentration remains within the detectable range.
	Improper sample storage or too many	Try to use aliquoted samples that have been

	freeze-thaw cycles	frozen for no more than 3 months; use aliquots to minimize repeated freeze-thaw cycles.
	There are interfering factors in the sample solution	While ensuring that the detected concentration falls within the detection range, increase the dilution factor to reduce interference; if the issue cannot be resolved, contact our technical staff for specialized adjustments.
	The content of the analyte in the sample is too low	Reduce the dilution factor or concentrate the sample before adding it
High background value	Incubation time/temperature does not match the instructions	Please strictly follow the instructions in the manual for the incubation reaction.
	The micro-plate was not covered with a sealing film before the reaction	During incubation, cover with a sealing film to prevent liquid evaporation and contamination.
	Abnormalities in the washing or sample-loading process may inadvertently introduce contamination	Use the washing solution provided with the kit, ensure the number of washes and the volume of the washing solution are sufficient, and make sure the wells are clean and free of residue after washing; ensure the laboratory work area is clean.
	Color shows without light protection / long duration	Color development should be done away from light, and the color development time should refer to the instructions.
The coefficient of variation (CV) is relatively high	The pipette is inaccurate	Use a calibrated pipette and operate it correctly
	Edge effect	Ensure that all reagents and micro-plates are at the same temperature
	Reagent mixing	Reagents from different batches should not be mixed.
Spike recovery exceeds the acceptable range	Matrix interference	Assess matrix effects through linear dilution and recovery experiments. Increase the dilution factor to reduce interference while ensuring the detected concentration falls within the detectable range; if the issue cannot be resolved, contact our technical staff for specialized adjustments.
	The selected spiking concentration is inappropriate	The spiked concentration is best set higher than the concentration of the target analyte in the actual sample; when selecting the spiked concentration at low levels, it should not be lower than the quantitation limit.