Ver202505

Instructions for Use of Host Cell Protein Residual Detection Kit for Pichia

Cat.No. HG-HCP005

Contents

1. Product Description

This kit employs a sandwich ELISA method for the detection of Pichia host cell protein (HCP) residues. A microtiter plate pre-coated with Pichia HCP capture antibodies specific binds HCPs present in samples or standards. After washing to remove unbound components, an enzyme-conjugated detection antibody is added. Subsequent washing removes excess detection antibody, followed by chromogenic substrate incubation and reaction termination. The measured absorbance (OD) correlates positively with HCP concentration in samples. By comparing sample OD values to the standard curve and applying the corresponding dilution factor, the residual Pichia HCP content is quantified.

Detection Range :1.5625 ng/mL　100 ng/mL

2. Application

Designed for quantitative detection of residual Pichia HCPs in intermediates, semi-finished products, and final biologics/pharmaceutical formulations.

3. Kit Specifications

Cat. No.	Specifications	Shelf Life
HG-HCP005	96T	12 months
HG-HCP005-S	48T	

4. Kit Components

Components	Specifications	Storage Conditions
Pichia HCP Standard	1 mL×8 vials	Unopened kits: Store at -20 , Opened kits: Store at 2-8
100×Anti-Pichia HCP-HRP	200 μL×1 vial	
Sample Diluent Buffer	15 mL×1 vial	
Antibody Dilution Buffer	15 mL×1 vial	
20×Wash Buffer	25 mL×2 vials	
Color Reagent A	6 mL×1 vial	
Color Reagent B	6 mL×1 vial	
Stop Solution	10 mL×1 vial	
Coated Plate	8 wells ×12 strips	
Sealing Film	4 pieces	

5. Storage Conditions and Shelf Life

This kit should be stored at -20 before the kit opening. The kit has a shelf life of 12 months from the date of manufacture. After opening, store the kit at 2-8°C and use within 30 days.

6. Definitions/Terminology

- OD: Optical Density
- CV: Coefficient of Variation, defined as the ratio of the standard deviation to the mean.

- ERC Extraction Recovery Control

7. Required Reagents, Consumables & Equipment

- ELISA Microplate Reader
- Microplate incubator/shaker (Provide incubation temperature)
- Vortex Mixer
- Deionized Water
- Absorbent paper
- Micropipettes and Pipette Tips

8. Operation Procedure

8.1. Assay Workflow Diagram

Total Duration: About 2 Hour and 50 Minutes

8.2. Preparation

8.2.1. Remove the kit from 2-8°C storage and equilibrate to room temperature (30 min) prior to use. Record the opening date on the kit when first opened and always use the earliest opened kit first. Kits from the same lot number may be used interchangeably; do not mix components from different lot numbers. Prepare the required number of ELISA strips for the current test. Return any unused strips to the foil sealed bag, and store at 2 8 °C for future use.

8.2.2. Wash Buffer (1×) Preparation: Dilute 20× Wash Buffer with deionized water at 1:19 ratio. If crystals are present in the 20 × concentrate, incubate at room temperature or 37°C with gentle agitation until fully dissolved before dilution.

8.3. Standard Curve Preparation: Pichia HCP Calibrators require no dilution and are ready for direct use.

8.4. Test Sample Preparation: Dilute samples with Sample Diluent Buffer to fall within the standard curve range. Mix thoroughly.

8.5. Extraction Recovery Control (ERC) Preparation: Combine 120 µL test sample with 120 µL of 25 ng/mL calibrator. Mix thoroughly.

8.6. Plate Washing: Wash the plate with 1 × Wash Buffer (300 µL/well), remove residual liquid by gently tapping the plate. Repeat for 3 cycles.

8.7. Sample Incubation: Add 100 μL of standard samples, test samples, and ERC samples to assigned wells. Seal plate with adhesive film, then incubate at 300 rpm and 37 for 1 hour. (*Note: Incomplete sealing may cause evaporation and data deviation*).

8.8. Plate Washing: : After incubation, equilibrate plate at room temperature for 3-5 min. After removing film and discarding the liquid, wash the plate with 1× Wash Buffer (300 μL/well). Aspirate the buffer and remove residual liquid by gently tapping the plate on absorbent papers. Repeat for 3 cycles. (*Note: Dispense buffer without touching well walls; Soak for 30 seconds , then gentle shaking; Use fresh* absorbent papers *sections for each dry step.)*

8.9. Detection Antibody Preparation: Dilute 100× Anti-Pichia HCP-HRP 1:100 with Antibody Dilution Buffer (e.g., 100 μL antibody + 9.9 mL buffer).

8.10. Antibody Incubation: Add 100 μL diluted antibody per well. Seal plate with adhesive film, then incubate at 300 rpm and 37 for 1 hour.Take out Color Reagent A and B from the refrigerator at 4 and leave them at room temperature.

8.11. Plate Washing: After incubation, equilibrate plate at room temperature for 3-5 min. After removing film and discarding the liquid, wash the plate with 1× Wash Buffer (300 μL/well). Aspirate the buffer and remove residual liquid by gently tapping the plate on absorbent papers. Repeat for 5 cycles.

8.12. Color Development: Mix Color Reagent A and B in a 1:1 volume ratio, add 100 μL/well to the microplate, seal the plate , and incubate at room temperature in the dark for 20 minutes.

8.13. Reaction Termination: Add 50 μL Stop Solution per well. Read plate immediately (*Note: It is recommended to set a 5 10 second shaking step in the microplate reader program.*).

8.14. Absorbance Measurement: Within 20 minutes of adding Stop Solution, measure absorbance at 450 nm with a reference wavelength of 630 nm using a microplate reader.

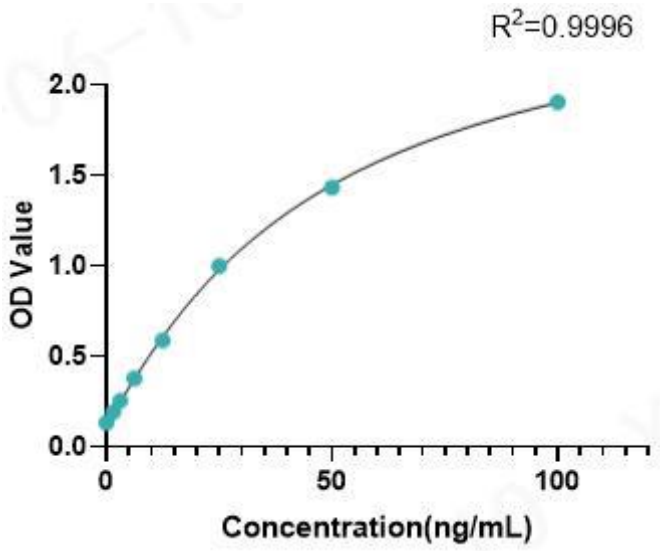
9. Result Analysis

9.1. Standard Curve Fitting: Plot the absorbance of the standards on the y-axis and their concentrations on the x-axis. Use a four-parameter logistic regression equation to fit the standard curve.

9.2. Schematic diagram

Concentration of standards(ng/mL)		OD Value 1	OD Value 2	CV%	Concentration (ng/mL)	Recovery Rate(%)
NTC	0	0.12680	0.13760	5.8%	0.00000	/
ST7	1.5625	0.19510	0.19100	1.5%	1.48865	95
ST6	3.125	0.25670	0.25180	1.4%	3.16688	101
ST5	6.25	0.37900	0.37570	0.6%	6.46435	103
ST4	12.5	0.58610	0.58920	0.4%	12.37827	99

ST3	25	0.97200	1.02780	3.9%	26.68734	107
ST2	50	1.42480	1.44140	0.8%	49.53991	99
ST1	100	1.88560	1.92340	1.4%	95.70033	96



9.3. Result Calculation

9.3.1. Calculation of Testing Example Residual (Pichia HCP) Concentration ng/mL

$$Pichia\ HCP\ Residual\ of\ Tesing\ Sample(\mathrm{ng/mL}) = Dilution\ factor \times Fitting\ residual\ Concentration\ of\ Testing\ Sample$$

9.3.2. ERC sample Recovery Rate (%) Calculation:

$$ERC\ Recovery\ Rate\% = \frac{(ERC\ Sample\ Concentration \times Total\ Volume)\text{-}(Test\ Sample\ Concentration \times Sample\ volume)}{ERC\ Sample\ Theoretical\ Concentration \times ERC\ Sample\ Volume} \times 100\%$$

10. Precautions

10.1. All components in the kit must be equilibration to room temperature (18 25°C) before use.

10.2. All reagents should be thoroughly mixed before use. Standards should be briefly centrifuged for 5 seconds to collect any liquid adhering to the tube wall or cap. After use, all reagents must be immediately returned to their specified storage temperature.

10.3. The kit must be used within its expiration date. A standard curve should be prepared for each assay. It is not recommended to mix reagents from different batch numbers.

10.4. When adding liquids to the ELISA plate, avoid touching the bottom of the wells to prevent damage to the coating. Change reservoirs and pipette tips between different samples and assay steps to avoid cross-contamination.

10.5. After washing the strip wells, tap them dry carefully to avoid detaching the strips. Do not reuse sealing membranes.

10.6. A black precipitate may appear during the color development step at high concentrations. This is a normal phenomenon and has no significant impact on final absorbance readings.

10.7. Ensure the detection wavelength and the chosen fitting equation are correct when reading absorbance values.

10.8. Optimal assay performance can only be ensured by strictly following the instructions and using all matched reagents provided in this kit.

10.9. Variability in results may be caused by multiple factors, including operator technique, pipetting method, washing procedure, reaction time or temperature, and storage conditions of the kit.

10.10. This kit is for research use only and is not intended for clinical diagnostic applications.

11. Troubleshooting

Seq. No.	Problem Description	Possible causes	Countermeasures
1	Standard Curve Gradient Issues	Inaccurate pipetting or liquid transfer	Check pipettes and pipette tips
		Incomplete microplate washing	Ensure proper wash cycles and sufficient wash buffer volume per well
2	Neither the standard melody nor the sample shows color or shows weak color	Insufficient incubation time	Ensure adequate incubation duration
		Incorrect reaction temperature	Use recommended incubation temperature
		Missing addition of a component, especially detection antibody or enzyme	Check experimental records and remaining reagents. Always verify reagent labels before each addition.
		Reagents are expired	Use only reagents within their expiration dates.
		Inactivation or loss of standard, antibody, enzyme, or substrate	Ensure proper storage. Replace with new standard, antibody, enzyme, or substrate if necessary.

		Delay between washing the plate and adding the next reagent	Add the next reaction solution immediately after washing and tapping the plate dry.
3	Weak or No Color Development	Inadequate vortexing during serial dilution of standards	Use vortex mixing during preparation and dilution of standards to ensure thorough mixing.
4	Low OD Readings	Incorrect microplate reader settings	Verify wavelength and filter settings on microplate reader
			Preheat instrument prior to readings as recommended
		Improper Plate Washing Operation: e.g., excessive washing cycles, prolonged incubation with wash buffer	Wash the plate according to the recommended procedure in the manual
5	High Coefficient of Variation (CV)	Incompatible Pipette and Tip	Replace with matching pipette tips.
		Pipette calibration issues	Conduct regular calibration and performance verification of pipetting

			equipment
		Check the Bottom of the Microplate	Inspect the bottom of the plate for any residual liquid or fingerprints.
		Inconsistent Pipetting Technique	Practice consistent pipetting techniques to ensure uniform handling.
		Abnormal Conditions Inside Wells	Ensure no foreign substances are present before sample addition and check for bubbles after pipetting.
6	High Background Value	Incomplete plate washing	Follow manufacturer's recommended washing protocol
			For automated washers: Verify all fluid ports are unobstructed
			For manual washing: Increase wash cycles as needed
		Contaminated common reagents: e.g. purified water	Replace with fresh, uncontaminated reagents
		Contaminated shared equipment e.g. pipette & centrifugate	Use dedicated pipettes with sterile filtered tips & equipment

		Cross-contamination from shared workspaces	Establish physically separated work zones for different procedures
		Incorrect reagent preparation	Prepare fresh solutions using proper dilution factors
		Overextended Incubation Time	Stop the reaction immediately using stop solution once sufficient color development has occurred; shorten incubation time if needed.
		Color Development Performed Without Light Protection	Ensure color development is carried out under light-protected conditions.
7	Significant Deviation Between Experimental Results and Reference Performance Parameters	Improper storage of the kit	Store all reagents according to the instructions provided in the manual.
		Expired reagents	Ensure that the kit and all components are within their expiration date.
		Failure to strictly follow the manual during operation	Perform hands-on training for laboratory personnel before the experiment to ensure proper execution.
			Strictly follow the manual for critical steps such as reagent concentration,

			sample volume, and incubation time; do not rely on estimated values.
8	Positive Result in Negative Control	Sample or reagent contamination, or improper pipetting leading to splashing and cross-contamination between adjacent wells	Replace reagents and handle samples carefully.
		Incomplete washing of the microplate	Ensure complete removal of antibody solution before washing. Fill each well fully with wash buffer to ensure thorough washing.
9	Large Variation in Calculated Sample Values Across Different	Strong matrix effect in the sample	Select two dilution gradients where the calculated sample values are close, especially for samples with high target protein concentration.

	Dilution Gradients		

12. References

- Pharmacopoeia of the People s Republic of China (2020 Edition)
- I ICH Guidelines Q6B: Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products

13. 13. Contact Information

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- Website: https://www.hillgene.com

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Appendix 1: Safety Precautions

- General Instructions

Failure to use this product in accordance with the instructions provided in this manual may result in personal injury or damage to instruments or equipment. Ensure that personnel using this product have received appropriate training in general laboratory safety practices and have reviewed the safety information provided herein.

(1) Before operating any instrument or equipment, read and understand all safety information provided in the user documentation from the respective manufacturer.

(2) Prior to handling any chemicals, read and understand all applicable Safety Data

Sheets (SDS), and wear suitable personal protective equipment (PPE), including gloves, protective clothing, and safety goggles.

- Biological Hazards

(1) Biological samples, including tissues, bodily fluids, infectious agents, and blood from humans or other animals, may pose a risk of transmitting infectious diseases. All procedures must be conducted in facilities equipped with appropriate safety equipment, such as certified biological safety cabinets. Additional safety measures include the use of personal protective equipment (PPE), such as gloves, lab coats, coveralls, shoe covers, boots, respirators, face shields, safety glasses, or goggles.

(2) Individuals working with potentially biohazardous materials must receive training in accordance with local regulations and institutional or corporate requirements prior to handling such materials.

- Hazardous Waste (from Instruments)

Waste generated by the instruments may present potential hazards. Follow the guidelines described under Biological Hazards to handle such waste safely.

Appendix 2: Related products For more products, please consult:https://www.hillgene.com

Category	Product name	Cat. No.
E. coli	E.coli Residual DNA Detection Kit (qPCR)	HG-ED001
	E.coli Residual DNA Fragment Analysis Detection Kit (qPCR)	HG-EF001
	E.coli Residual Total RNA Sample Preprocessing Kit	HG-CL300
	E.coli Residual Total RNA Detection Kit (RT-PCR)	HG-ER001
	E.coli HCP ELISA Detection Kit (2G)	HG-HCP002-2G
293 cells	293T HCP ELISA Detection Kit	HG-HCP001
	HEK293 cell Residual DNA Detection Kit (qPCR)	HG-HD003
	HEK293 Cell Residual DNA Fragment Analysis Detection Kit (qPCR	HG-HF002
	293T Cell Residual DNA Detection Kit (qPCR)	HG-HD004
	293T Cell Residual DNA Fragment Analysis Detection Kit (qPCR	HG-HF003
Pichia	Pichia Residual DNA Detection Kit (qPCR)	HG-PD001
Antibiotic	Kanamycin ELISA Detection Kit	HG-KA001