HEK 293 Host Cell Protein ELISA Kit, G3

Applicable to HEK293-related lines (293T, 293F, etc.)

48 Wells

Catalogue Number: HH-H0019-3A1-48T

Valid Period: 6 months

ELISA Kit for the quantitative Measurement of HEK 293 HCP Residues in Cell Culture Supernatants, Protein Purification Process, and End-Product

FOR RESEARCH, DEVELOPMENT AND MANUFACTURING USE ONLY!
NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

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1. BACKGROUND

This kit is intended for the quantitative detection of host cell protein (HCP) residues in biopharmaceuticals produced using 293 cell-based expression systems, including HEK293 and 293T cell lines.

293 cell lines are widely utilized for the production of therapeutic proteins. During target protein expression, inherent cellular apoptosis occurs concomitantly. Published data indicate that over one thousand distinct host cell proteins are released into the culture medium upon cell lysis. A substantial proportion of these proteins exhibit strong immunogenicity, which can trigger adverse toxic or immune responses. Such responses jeopardize the safety and quality of biopharmaceutical products while posing risks of potential biological contamination. Consequently, the removal of these hazardous impurities represents a core objective of downstream biomanufacturing processes. Therefore, it is imperative to minimize residual HCP levels to the lowest feasible extent. In the development of downstream purification processes, a scientifically validated and reliable method for quantifying HCP concentrations in both finished and semi-finished products is indispensable. Enzyme-Linked Immunosorbent Assay (ELISA), renowned for its ultra-high sensitivity, has been established as the gold standard for HCP detection by regulatory authorities worldwide, including the FDA, EMA, NMPA, and ICH.

2. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the solid phase sandwich Enzyme-Linked Immunosorbent Assay. The microplate has been pre-coated with a capture antibody. The antigen to be measured and the HRP-conjugated detection antibody are then added to the microplate wells sequentially to form a capture antibody-antigen-detection antibody complex. After washing, the conjugates uninvolved in the reaction are removed, and then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP and finally turns yellow immediately after adding the stop solution. The color of the TMB substrate positively correlated with the antigen bound in the initial steps. Measure the absorbance (OD value) at 450nm using a microplate reader, and create a standard curve along with the corresponding concentrations. Then, by inputting the OD

values of the samples into the standard curve equation, calculate the concentration of the target protein in the sample.

3. KITS' ADVANCEMENT

1) High Coverage: The capture and detection antibody exhibit a strong capacity for recognizing host cell proteins (HCPs), with a coverage rate of over 80% and the manufacturing process demonstrated excellent process stability across multiple batches.
2) High Antibody Titers: The antibodies used in the reagent kit are tested using the indirect method with ELISA, and the results indicate a titer of 10^6.
3) High Sensitivity: Serum antibody purification employs affinity purification to remove non-specific antibodies to the greatest extent.
4) High Stability: The production process uses a broad-spectrum protein stabilizer and microplate processing technology to enhance the stability and repeatability of the standard and microplate result.
5) Applicability: The kit was evaluated under different reaction temperatures (20-30°C) and reaction time variations (±10 minutes), demonstrating excellent reproducibility of test results.
6) Optimal Diluent Buffer: Using an optimized dilution solution can reduce non-specific adsorption during the sample detection process, resulting in very low background coloration that facilitates the observation of the concentration of the samples.

4. MATERIALS (Note: Store at 2-8°C)

	Reagents	Specification	Quantity
1	Pre-Coated Microplate (Detachable)	48 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500μg/mL	30μL	1 tube
3	Detection antibody (100×)	100μL	1 tube
4	TMB Substrates	10mL	1 vial (Avoid Light)
5	Stop Solution	10mL	1 vial
6	Wash Solution (20×)	10mL	5 vials
7	Diluent Buffer (10×)	10mL	1 vial

8	Plate Sealer		4 pieces
9	Instruction Manual		1

5. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) 10-1000μL Pipettor
2) Multichannel Pipettor
3) 1L sterilized Deionized Water or Ultrapure Water
4) Sterilized EP Tubes
5) Absorbent Paper
6) Microplate Reader
7) High-speed Centrifuge
8) Mini Centrifuge
9) Microplate Washer or Washing Bottles
10) Data Analysis and Graphing Software

6. PREPARATION BEFORE ASSAY

Please read the kit instruction manual carefully. All reactions are performed at room temperature (20-25°C, the same applies hereinafter).

7. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30 minutes before using.
2) The preparation of Wash Solution (1×): Add the contents of five 10mL of Wash Solution (20×) into 950mL of deionized water or ultrapure water to prepare 1000mL of Wash Solution (1×). If crystals have formed in the concentrate, allow it to reach room temperature and mix gently until the crystals are completely dissolved.
3) The preparation of Diluent Buffer (1×): Add 10mL of Diluent Buffer (10×) into 90mL of deionized water or ultrapure water to prepare 100mL of Diluent Buffer (1×). If crystals have formed in the concentrate, allow it to reach room temperature and mix gently until the crystals are completely dissolved. The buffer is used as the Diluent Buffer for standards, samples and detection antibodies.

8. ASSAY PROCEDURE

1) Remove the microplate from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4°C. Then put the ready-for-use strips on a clean microplate frame, and start the experiment after the strips returned to room temperature (Note: The microplate frame can be reused). The standard may stick to the tube wall due to transport turbulence. Before use, gently shake it or centrifuge for about 2 seconds.

2) Prepare Standards: When preparing the standards, label 8 tubes as IS0, S1, S2, S3, S4, S5, S6, S7 and add the following volumes of Diluent Buffer (1×) to each tube: IS0 (98μL), S1 (460μL), S2 to S6 (each 250μL), S7 (350μL).

① Add 2μL of Standard (Stock Solution - 500μg/mL) into the IS0. Mix gently by pipetting up and down twice, then invert to mix thoroughly.

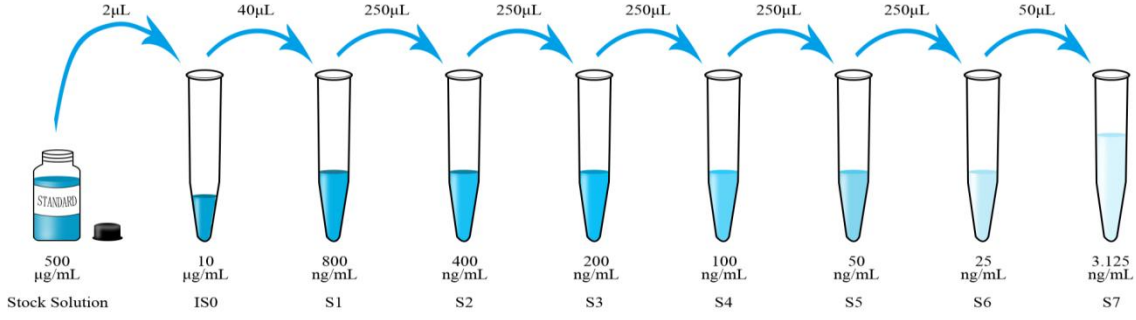
(All subsequent mixing steps should be performed in the same manner - do not vortex or use any vigorous mixing method.)

② Transfer 40μL of the resulting IS0 (10μg/mL) to the tube S1. Mix gently by pipetting up and down twice, then invert to mix thoroughly.

③ Transfer 250μL of S1 (800ng/mL) to S2, mix gently; then transfer 250 μL of the resulting S2 solution (400ng/mL) to S3, mix gently. Continue this 2-fold serial dilution sequentially through S6 to achieve a final concentration of 25 ng/mL.

④ Transfer 50μL of the S6 (25ng/mL) to S7 and mix gently by pipetting up and down twice, then invert to mix thoroughly.

NOTE: There are 7 points of diluted standards, S1: 800ng/mL, S2: 400ng/mL, S3:200ng/mL, S4: 100ng/mL, S5: 50ng/mL, S6: 25ng/mL, S7: 3.125ng/mL.



3) Sample Addition: Add 100μL of standards or test samples to the microplate wells. For the Blank Control, add 100μL of Sample Diluent only.
4) Seal the microplate with the plate sealer and incubate at room temperature on a shaker for 1.5 hours.
5) Automated Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000mL Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.
- Fill each well with 300μL Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times.

  Note: There should be no moisture appears in the fifth washing step.

6) Prepare Detection Antibody: Dilute 100μL of Detection antibody (100×) to its working concentration (1×) with 10mL Diluent Buffer and mix gently. Add 100μL of the diluted Detection Antibody to each well, seal the plate with the plate sealer, and incubate at room temperature on a shaker for 1.5 hours.
7) Washing Step: Repeat the same procedure as step 5.
8) Add 100μL TMB substrate to each well. Cover the plate with the plate sealer, incubate at room temperature for about 15 minutes. If the color is light, the reaction time can be extended appropriately, but not more than 30 minutes.

9) Add 50μL of Stop Solution to each well to stop the reaction.

10) Run the microplate reader and conduct measurement at 450nm.

11) Data analysis: Four parameter curve fitting is recommended.

9. NOTES

1) Sample Preparation

a) After collection, the samples should be aliquoted and stored at -20°C (less than 3 months) or -80°C (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2-8°C.

b) Ensure the frozen samples are completely thawed before use (do not thaw by heating). Mix thoroughly using a pipette or vortex mixer. If precipitates are present in the sample, they may interfere with the ELISA results; centrifugation is recommended to remove the precipitates.

c) Some chemical solvents may interfere with this experiment, such as SDS, Triton, etc.

d) It is recommended that all standards and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) The process of protein purification is often accompanied by complex buffer solutions. To exclude matrix effect, it is recommended to perform spike recovery test when using different buffers for the first time. The acceptable spiking recovery rate is 80-120%. High salt, low PH, polysaccharide, organic solvents, and detergents can result in lower recovery rates. The specific procedure is to add Standard S1(800ng/mL) Diluent Buffer into a buffer solution in a 1:1 volume ratio (e.g. add 50μL of standard S1(800ng/mL)/Diluent Buffer to 50μL of the buffer solution). For calculation of recovery rate, subtract the background concentration before spiking from the concentration after spiking, then divide the result by the theoretical concentration to obtain the spike recovery rate.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, use disposable sterilized tips during the experiment.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. If there are too many samples, a multichannel pipettor is recommended.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

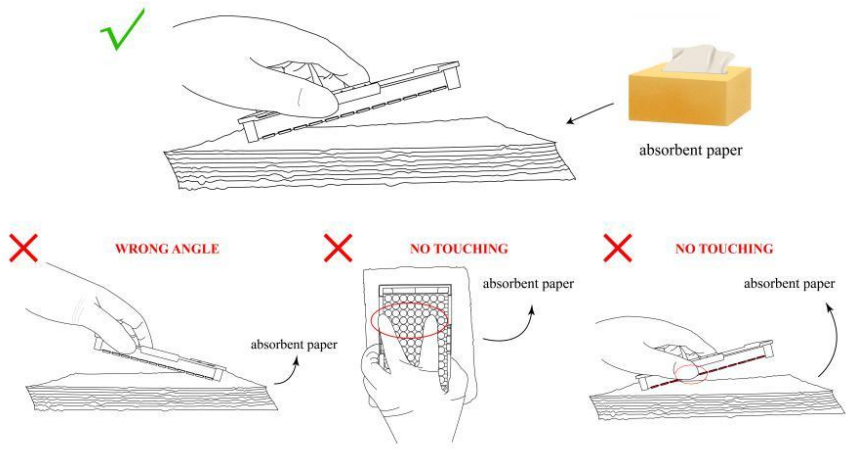
e) TMB is easily contaminated. Please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is diluted sulfuric acid. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geqslant 0.95$.

i) Schematic diagram of tapping the plate:



10. CALCULATION OF RESULTS

1) Average the duplicate readings for each standard and sample.

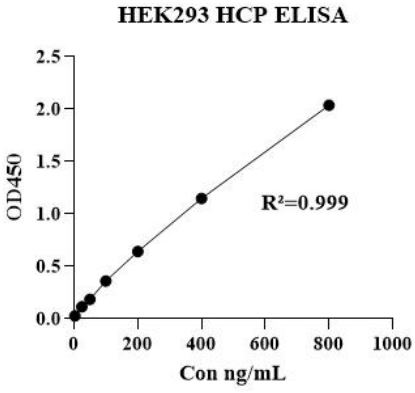
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four-parameter logistic

(4-PL) curve-fit.

3) Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.

4) Standard curve is for demonstration only.

APPENDIX 1: EXAMPLE OF STANDARD CURVE



	Con ng/mL	OD450
S1	800	2.038
S2	400	1.148
S3	200	0.643
S4	100	0.361
S5	50	0.187
S6	25	0.116
S7	3.125	0.029

11. QUALITY CONTROL

1) Intra Variation%: 7-9

   Inter Variation%: 7-9

2) Sensitivity:

   LOD: 0.39ng/mL

   LOQ: 3.125ng/mL

12. FAQs & TROUBLESHOOTING

If the experimental results are abnormal, promptly take photos to record the color development results, fully retain the unused strips and reagents, and contact technical support. In addition, you may refer to the troubleshooting information provided below to identify the root cause of the problem.

Problem Description	Possible Causes	Corresponding Solutions
Poor Standard Curve	Incorrect dilution of standards	Dilute the standard curve according to the specified ratio.

	Inaccurate pipetting or sample loading	Check the pipette and pipette tips.
	Incomplete washing of the microplate	Ensure the required number of washing cycles and the volume of washing solution per well.
Weak or Absent Color Development	Insufficient incubation time	Ensure adequate incubation time.
	Incorrect experimental temperature	Use the recommended incubation temperature.
	Insufficient reagent volume or missing addition	Check the pipetting and sample loading process to ensure all reagents are added in sequence with sufficient volume.
	Substrate solution not equilibrated to room temperature	Allow the TMB substrate to stand at room temperature for more than 30 minutes before color development.
Low OD Value Reading	Incorrect microplate reader settings	Verify the wavelength and filter configuration on the microplate reader. Preheat the microplate reader in advance before reading.
High Coefficient of Variation (CV)	Incorrect sample loading	Check the sample loading operation.
	Contamination on the microplate bottom	Inspect the microplate bottom for residual liquid and fingerprints.
	Foreign objects or air bubbles in wells	Confirm no foreign objects in wells before sample loading and no air bubbles after loading.
	Unsealed or incompletely sealed plate during incubation	Seal the plate with plate sealer.
High Background Value	Incomplete washing of the microplate	Wash the plate according to the method recommended in the instruction manual.
		If using an automated plate washer, check for clogs in all liquid inlets and waste outlets.
		For manual washing, appropriately increase

		the number of washing cycles.
		Inadequate or missed washing will result in high background.
	Incorrect incubation time or temperature	Operate strictly in accordance with the instruction manual.
	Contaminated consumables	Ensure the tubes, pipette tips and other consumables are clean.
	Contaminated washing solution	Prepare fresh washing solution.
	Contaminated substrate solution	The substrate solution is inherently colorless. Ensure the substrate is not contaminated by metal ions or oxidizing agents before use and store it away from light.
Low Sensitivity	Improper kit storage	Store all relevant reagents according to the requirements in the instruction manual.

13. CONTACT US

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