CHO Host Cell Protein ELISA Kit, G1

96 Wells

Catalogue Number: CH-K0018-1

Valid Period: 6 months

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. APPLICATION

CHO Host Cell Protein ELISA Kit is for research use only, it should not be used in clinical diagnostic procedures. The kit is intended for the quantitative determination of host cell protein impurities in Cell culture supernatants or host cell protein (HCP) contamination in end-product expressed by Chinese Hamster Ovary (CHO) expression systems.

2. BACKGROUND

CHO cell lines are widely used to express therapeutic proteins that, while expressing the protein of interest, undergo concomitant apoptosis. Data show that up to a thousand host proteins are released into the culture medium after cell disruption, and a significant proportion of them are strongly immunogenic, resulting in adverse toxicity or immune reactions that jeopardize product safety and quality and cause potential biological contamination. One of the aims of downstream processes in the production of biological medicinal products is to remove these potential hazards.

Therefore, it is highly necessary to reduce host cell protein (HCP) residues to a minimum level. In developing a process for downstream purification, it is necessary to have a scientific and sound method for determining the concentration of HCP in the finished product or semi-finished product, and the enzyme-linked immunosorbent assay (ELISA) has a very high sensitivity, so it has been set as the gold standard for HCP detection by regulatory authorities..

3. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the solid phase sandwich Enzyme Linked Immunosorbent Assay. The microtiter plate has been pre-coated with capture antibody. The antigen to be measured and the HRP-conjugated detection antibody are then added to the microtiter plate 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 to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with the antigen bound in the initial steps. The color is measured by spectrophotometrically with wavelength of 450nm. The concentration of ErbB2 in samples is then determined by comparing the O.D. of the samples to the

standard curve.

4. KITS' ADVANCEMENT

1) High Coverage: It is able to detect a broad range of HCP impurities. The capture and detection antibody are both sourced from rabbit, it has a broader ability ro recognize HCP that sourced from other species (e.g.,goats). And the species showed little interindividual variation and are more stable in production process
2) High Antibody Titers: The antibodies used in the kit that have been tested with sandwich ELLISA for titers of at least 10^6 during the animal immunization phase.
3) High Sensitivity: Serum antibody purification adopts the most advanced preparation process in the industry, AAE method, 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 increase the thermal stability of standards and microplates and the reproducibility of results.
5) Optimal Diluent Buffer: Use the optimized diluent buffer to reduce non-specific adsorption during sample detection. The low background is more advantageous for observing the concentration of the sample.

5. MATERIALS (Note: Storage at 2-8℃)

	REAGENTS	SPECIFICATION	QUANTITY
1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution) -500ug/ml	10ul	1 tube
3	Detection antibody (1400×)	15ul	1 tube
4	TMB Substrates	10ml	1 vial (Avoid Light)
5	Stop Solution	10ml	1 vial
6	Wash Solution (100×)	10ml	1 vial
7	Diluent Buffer (10×)	10ml	1 vial
8	Diluent Ingredient	0.5g	1 tube

9	Plate Sealer		4 pieces
10	Instruction Manual		1

6. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) Precision pipettors and disposable tips to deliver 10-1000 µl.
2) A multi-channel pipette is desirable for large assays.
3) 1L sterilized deionized water or hyperpure water.
4) Sterilized EP Tubes
5) Absorbent Paper
6) Microplate reader capable of measuring absorbance at 450 nm.
7) High-speed Centrifuge
8) Automated Microplate Washer and Washing Bottle.
9) Data analysis and graphing software.

7. SPECIMEN COLLECTION AND STORAGE

Cell culture supernatant - Centrifuge cell culture supernatant at 1000×g (or 3000 rpm) for 15 minutes, and collect the supernatant for immediate assay or store samples at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Samples containing a visible precipitate must be clarified prior to use in the assay.
- Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
- Do not use heat-treated specimens.

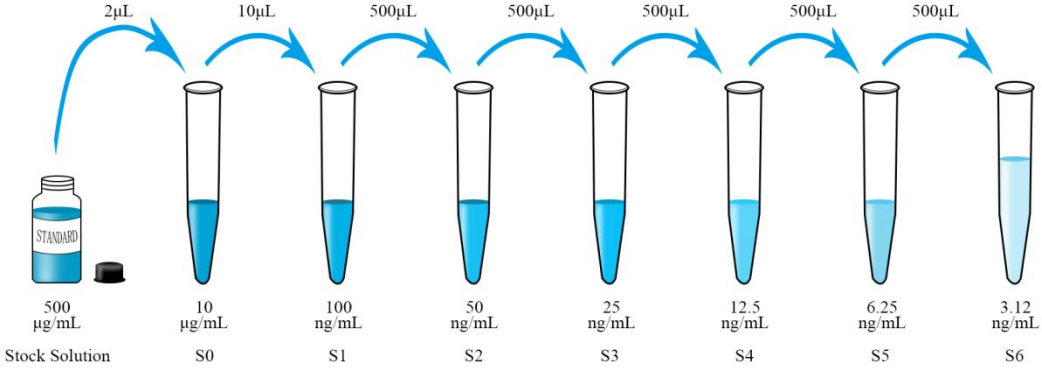
8. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.
2) The prepration of Wash Solution (1×): Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C.
3) The prepration of Diluent Buffer (1×): Dilute 10 mL of Diluent Buffer (10×) with 90 mL of deionized water to prepare 100 mL of Diluent Buffer . If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Then add 0.5g Diluent Ingredient into 100ml Diluent Buffer, mix well as the final Diluent Buffer (1×) for standards, samples and detection antibodies.

9. ASSAY PROCEDURE

1) Remove the microtiter plates from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at °C. Then put the ready-for-use strips on a clean micropore plate 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, so it shall be slightly shaken up before use, or centrifuged on the centrifuge for about 2 seconds.
2) Prepare Standards: The concentration of Standard (Stock Solution) is 500ug/ml. Add 2ul of Standard (Stock Solution) into 98ul Diluent Buffer (1×) as the S0=10ug/ml, then add 10ul of S0 (10ug/ml) into 990ul Diluent Buffer (1×) as the S1=100ng/ml. After that, prepare 500 ul Diluent Buffer (1×) for each tube from S2 to S6. Then add 500ul of S1 into S2, and then produce a 2-fold dilution series (see below). Secure the desired plate in the holder then add 100ul of diluted standards or 100ul (diluted or undiluted) samples to appropriate wells. For the Blank Control test, we recommend add 100ul Diluent Buffer (1×) to the well.

   NOTE: There are 6 points of diluted standards, S1: 100ng/ml, S2: 50ng/ml, S3: 25ng/ml, S4: 12.5ng/ml, S5: 6.25ng/ml, S6: 3.12ng/ml.



3) Cover the plate with sealer, put it on the oscillator to mix and incubate for 90 mins at room temperature.

4) Automated Washing:

- Put 1000 ml 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 1000 ml 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 300ul 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.

5) Prepare Detection Antibody: Extract 8ul of detection antibody (1400×), and add it into 11.2ml Diluent Buffer (1×) to reach its working concentration (1×) and mix gently. Add 100ul of above diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 90 mins at room temperature.

6) Washing Step: Repeat the same procedure as step 4.

7) Add 100ul TMB substrate to each well. Put it on the oscillator to mix for 30s. Cover the plate with a sealer, incubate at room temperature for about 10 mins. (Avoid sunlight). If the color is light, the reaction time can be extended appropriately, not more than 30min. Note: Please set the speed of the oscillator at 100rpm or less. Liquid spills will effect the OD. The coloring time varies in different experimental conditions (temperature, humidity, ect.).

8) Add 50ul of Stop Solution to each well to stop the reaction. Put it on the oscillator to mix well for 30s or mix the liquid by gently tapping the side of the plate.

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

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

10. NOTES

1) **Sample Preparation**

   a) After collection, the samples should be aliquoted and stored at -20℃ (less than 3 months) or -80℃ (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) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

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

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, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

   c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10

minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

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.

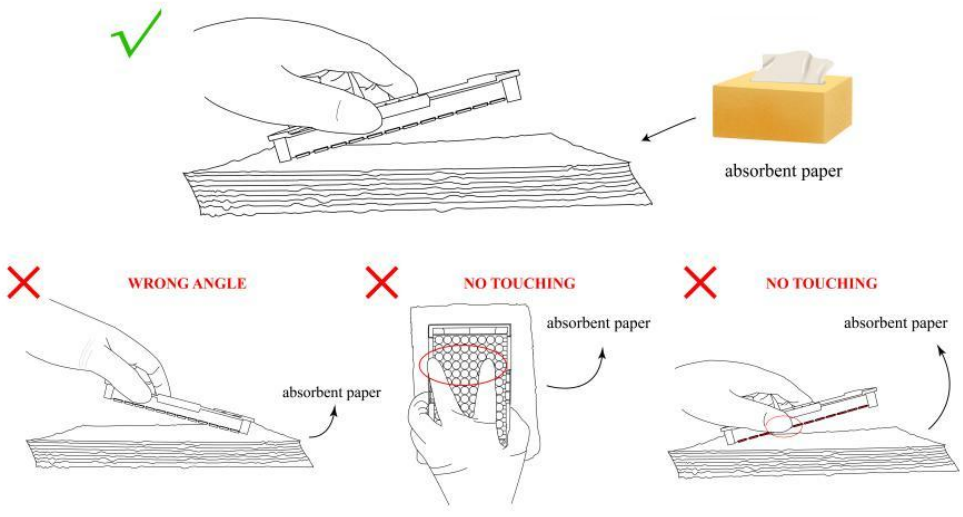
e) TMB is easily contaminated. Please protect it from light and metal.

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 acid solution. 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:



11. CALCULATION OF RESULTS

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

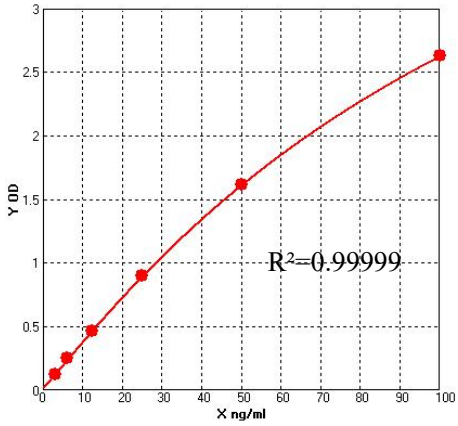
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y)

axis against the concentration on the horizontal (X) axis, and generate a four paramater 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



ng/ml	OD450nm
100	2.626
50	1.612
25	0.897
12.5	0.467
6.25	0.249
3.125	0.126
0	0.021

12. QUALITY CONTROL

1) Intra Variation%: 3.1-4.5

2) Inter Variation%: 6.0-9.1

3) Recovery%: 95-110

4) Linearity:

Diluent ratio	Range %
1:2	98.9-103.3
1:4	95.1-103
1:8	93.5-104.5
1:16	94.1-115.4
1:32	100-109.4

5) Sensitivity:

LOD: 0.2 ng/ml

LOQ: 3 ng/ml

13. CONTACT US

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