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03/18

CHO Host Cell Proteins Elisa kit

(Catalog # E4537-100 ; ; Storage at 2-8°C)

I. Introduction:

CHO cell line is widely used in the expression of therapeutic protein , which is a cost effective method for production of commercial quantities of a drug substance. One of the goal in designing a downstream process for biopharmaceutical products is to remove any possible contaminants, including host cell proteins. HCPs can still be present at significant concentrations following several purification steps and can even be co-purified and concentrated with the drug substance itself. HCP in the products will result in adverse toxic or immunological reactions which compromise product safety and quality. This it is desirable to reduce the host cell protein contamination to the lowest level. Therefore, during the development of a downstream process, suitable assays must be available to determine the amount of HCP present.. BioVision's CHO HCP ELISA kit is a sandwich ELISA assay for the quantitative measurement of CHO HCP in Human serum, plasma and cell culture supernatants. The density of color is proportional to the amount of CHO HCP captured from the samples.

II. Applications:

- Detection Range: 100 - 0.1 ng/ml
- No obvious cross reactivity has been observed between CHO HCP and Vero, E.coli and Yeast HCP.
- This ELISA kit is used for in vitro quantitative determination of CHO HCP in samples.
- Sensitivity: 0.31 ng/ml

III. Sample Type:

- Cell lysate
- Other biological fluids

IV. Kit Contents:

Components	E4537-100	Part Number
Micro ELISA Plate	8 X 12 strips	E4537-100-1
Detection Antibody	120 µl	E4537-100-2
Enzyme Conjugate (100X)	120 µl	E4537-100-3
Standard (100X)	10 µg/ml	E4537-100-4
Diluent	100 ml	E4537-100-5
TMB Substrate	12 ml	E4537-100-6
Wash Solution (100X)	10 ml	E4537-100-7
Stop Solution	10 ml	E4537-100-8

V. User Supplied Reagents and Equipment:

- Incubator (37°C)
- Microplate reader capable of measuring absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:

The entire kit may be stored at 4°C in dark for up to 12 months from the date of shipment. Avoid freeze-thaw cycles
Detection antibody- Dilute the Detection antibody (100×, 120uL) with 12 mL diluent. This working solution should be used only at the day of preparation.
Wash Solution: Dilute the wash solution concentrate (100X, 10 ml) in 990 ml water.
Enzyme concentrate- Dilute the Enzyme concentrate (100×, 120uL) with 12mL diluent. This working solution should be used only at the day of preparation.
Bring all kit components and samples to room temperature (20-25 °C) before use.

VII. Assay Protocol:

1. **CHO - HCP standard:** The standard concentrations of the CHO HCP ELISA should be prepared from the respective CHO HCP standard of the kit immediately before performing the assay
   a. Eight ELISA standards (S1-S8) are prepared in dilution buffer covering the assay working range (about 0.2ng/mL to 100 ng/mL CHO-HCP) according to the following dilution scheme. the standards should be used only at the day of preparation.

Standard ID	Concentration (ng/mL)	Volume Antigen(uL)		Volume Assay Buffer (uL)
			of	
Master standard	10.000	9	Master St.	891
S1	100	300	S1	600
S2	33	300		600
S3	11	300		600
S4	3.6	300		600
S5	1.2	300		600
S6	0.4	300		600
S7	0.1	300		600
S8				

2. Secure the desired numbers of coated wells in the holder then add 100 uL of Standards or Samples to the appropriate well in the antibody pre-coated Microtiter Plate. Cover and incubate the plate for 2 hours at 37°C.
3. Wash the microtiter plate using one of the specified methods indicated below

a. Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Fill in each well completely with 1× wash solution, and then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure five times for a total of FIVE washes. After washing, invert plate, and blot dry by hitting the plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame. Complete removal of liquid at each step is essential to good performance.

b. Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 uL/well/wash) using an auto washer. After washing, dry the plate as above. It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.

4. Add 100 uL of Detection Antibody to each well. Mix well. Cover and incubate the plate for 1.5 hour at 37°C.

5. Wash the microtiter plate five times as above.

6. Add 100 ul of Enzyme conjugate to each well. Mix well. Cover and incubate the plate for 45 min at 37°C.

7. Add 100 uL Substrate Solution to each well including control wells, subsequently. Cover and incubate for 10-15 minutes at 37°C. (Avoid light)

8. Add 100 uL of Stop Solution to each well including control wells. Mix well.

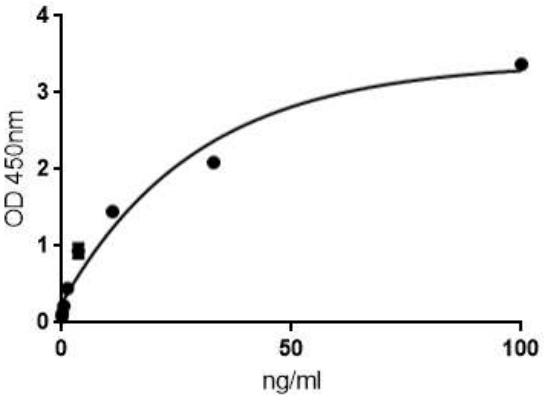
9. Determine the Optical Density (O.D.) at 450 nm using a microplate reader immediately.

10. Result Calculation:

a. First, average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.

b. 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 draw a best fit curve e using graph paper or statistical software to generate a linear regression, four parameters logistic (4-PL) curve-fit, or curvilinear regression of second degree. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.

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



Sample Standard Curve for CHO HCP ELISA Kit. (E4537-100)

Specificity /Cross-Reactivity:

Sample	Mean OD	Con calculated(ng/ml)	Cross reactivity（%）
Vero HCP	0.1375	1.22	0.08
Yeast HCP	0.1365	1.07	0.10
E.coli HCP	0.1375	1.22	0.07

VIII. Related Products:

Gentamicin (serum/urine) ELISA Kit (K4315)
QuickDetect™ E. Coli Protein (Human) ELISA Kit (E4451)
Ampicillin ELISA Kit (E4350)
QuickDetect™ BSA (Human) ELISA Kit (E4466)
Kanamycin ELISA Kit (K4210)

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