CHO HCP ELISA Kit

DD8102-P1

Vazyme

Instruction for Use

Version 25.1

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For Research Use Only. Not for use in diagnostic procedures.

01/Product Description

01-1/Intended Use

This kit is designed for the detection of host cell protein residues in process samples of biologics (such as antibodies, recombinant proteins, vaccines, etc.) expressed in CHO cell lines.

01-2/Detection Principle

This kit employs a fed-batch culture process using CHO to immunize goats for the production of HCP-specific antibodies. The kit is based on a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to detect residual CHO-HCPs in biologics process samples. The wells of the ELISA plates are pre-coated with Anti-CHO-HCPs goat polyclonal antibodies. CHO-HCPs calibrators and test samples are added to the solid-phase antibody-coated wells, followed by the addition of the detection antibodies-HRP conjugate. Through a one-step incubation, any residual CHO-HCPs present in the test samples are captured by the solid-phase antibodies and simultaneously detected by the detection antibodies, forming a "coated antibody-(CHO-HCPs antigen)-detection antibodies-HRP conjugate" complex. Unbound substances are removed by washing, and TMB substrate is added for color development. The reaction is then stopped, and the absorbance is measured at dual wavelengths of 450 nm and 630 nm. The absorbance of the samples is positively correlated with the residual amount of CHO-HCPs in the process samples. The concentration of CHO HCPs in the samples can be calculated using a dose-response curve.

02/Components

	Components	DD8102-P1 (96 tests)
	Pre-coated 96-Well microplate (coated Anti-CHO-HCPs)	12 × 8, 96 wells
	Sample diluent	2 × 30 ml
	Standard 1 (125 ng/ml)	1 ml
	Standard 2 (100 ng/ml)	1 ml
	Standard 3 (40 ng/ml)	1 ml
	Standard 4 (12 ng/ml)	1 ml
	Standard 5 (3 ng/ml)	1 ml
	Standard 6 (1 ng/ml)	1 ml
	Standard 7 (0 ng/ml)	1 ml
	QC sample (50 ng/ml)	1 ml
	Detection antibody-HRP conjugate	12 ml
	Concentrated washing solution (20 ×)	30 ml
	TMB substrate	12 ml
	Microplate sealing film	3

Note: This test kit cannot be mixed with other commercial test kits.

03/Storage

1. Store at 2 ~ 8°C and ship on ice pack.
2. After unsealing the pre-coated ELISA plate, the remaining coated strips should be sealed and stored at 2 ~ 8°C, and should be used within the validity period.
3. Other components of the test kit should be put back to 2 ~ 8°C in time after use, and should be used within the validity period.

04/Applications

1. No special treatment is required for test samples. It is recommended to validate the suitability of the kit by testing at appropriate dilution ratios.
2. Within the quantitative detection range of the kit, this may indicate the presence of a hook effect if the HCP concentration of an undiluted sample is lower than that of a diluted sample. It is recommended to perform gradient dilution of the sample for further testing.
3. Sediment and suspended matter in the sample may affect the test results, which should be removed by centrifugation at 6,000 × g 10 min.
4. Before the detection, the temperature of samples must be fully recovered to the room temperature (18 ~ 28°C). Frozen samples should be completely thawed, rewarmed and mixed evenly before use, and repeated freezing and thawing are not allowed.

05/Self-prepared Materials

Reagents and consumables that are required but not provided:

>Deionized water or distilled water

>Oscillator

>Microplate washer

>Stop solution (component concentration): 0.5 M Sulfuric acid, 1.2 M Citric acid monohydrate

>Micropipette and matched sterilization tip

>Incubator or water bath

>Microplate reader

>Sample adding slot

>Absorbent paper

06/Notes

1. Please read the Instruction for Use carefully before operation, and carry out the experimental operation in strict accordance with the instructions of the test kit.
2. The experiments must not be performed in harsh environmental conditions (such as the environment containing 84 disinfectant, sodium hypochlorite, acid, alkali or acetaldehyde and other corrosive gases of high concentration, and dust), and the laboratory disinfection should be carried out after experiment.
3. The kit should be balanced at room temperature before opening and using, and the reagent should be shaken thoroughly before use. The Instruction for Use should be strictly followed during storage and usage of each component, and it should not be changed or diluted without permission. Before use, the validity period and packaging of the kit should be strictly checked. It must not be used for experiments if the validity period has expired or the package has been damaged. After the reagents need to be used within the validity period, the remaining reagents should be sealed in time and kept according to the Instruction for Use.
4. The pre-coated ELISA plate can be disassembled, and after taking out the required number of plates each time, the remaining unused plates must be put back into the self-sealing bag and stored at 2 ~ 8°C for later use. When disassembling the plate, the bottom of the well must not be touched, to avoid fingerprints or scratches that may affect the subsequent reading. After washing the plates, it must not be left for too long to dry and deactivate, and then the next step should be continued immediately.
5. When adding samples, the production of bubbles should be avoided, and the gun tip should not touch the bottom of the plate to avoid affecting the reading value due to scratches.
6. The Microplate sealing film must not be re-used. The reagent components of different batch numbers should not be mixed, and the tips of micropipettes should not be mixed to avoid cross-contamination.
7. If the concentrated washing solution is crystallized, it should be placed at 37°C until dissolved before use. When washing, each well should be filled with washing solution to prevent the substrate marked by free enzyme at the orifice from being washed clean. They should be washed thoroughly, and too much solution should not be added to avoid serial flow. The liquid in the well should be rotated and dried every time, and the liquid in the well should be patted and dried at last (the microplate washer is recommended for plate washing).
8. The results should be read within 15 min after the termination of the reaction.
9. The operator should wear the disposable gloves and protective articles as specified by the laboratory in operation, and upon detection, the harmless disposal of waste liquid and appliances must be performed according to relevant regulations of the local government and state.

07/Inspection Method

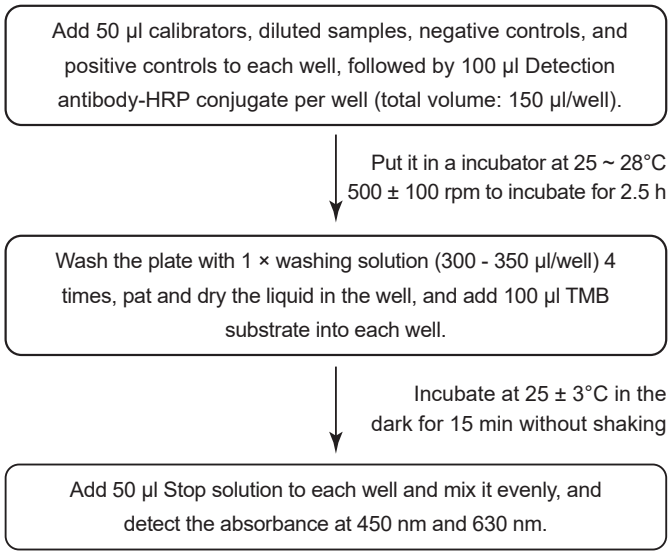
07-1/Experiment Preparation

1. All reagents and samples should be balanced to room temperature (at least 30 min).
2. Adjust the temperature of the thermostatic incubator oscillator to the required range.
3. Preparation of 1 × washing solution: Dilute the Concentrated washing solution (20 ×) 20 times with deionized water or distilled water and mix them evenly for later use, for example, dilute 30 ml Concentrated washing solution (20 ×) with 570 ml deionized water or distilled water.

07-2/Experiment Process

1. Sample adding: According to the plate layout, add 50 µl of calibrators, diluted samples, negative controls, and positive controls to the corresponding wells, followed by 100 µl of Detection antibody-HRP conjugate per well. Replicates are recommended.
2. Incubation: After sealing the plate with a Microplate sealing film, put it in a incubator at 25 ~ 28°C and 500 ± 100 rpm to incubate for 2.5 h.
3. Washing: After the incubation, carefully remove the Microplate sealing film and discard the liquid in the wells. Add at least 300 µl of 1 × washing solution into each well, and discard the 1 × washing solution after standing for 10 - 30 s. Wash the plate 4 times continuously, and try to remove the residual liquid at the last time.
4. Color development: Add 100 µl TMB substrate into each well, seal the plate with a Microplate sealing film, and then put it into a incubator at 25 ± 3°C to incubate in the dark for 15 min.
5. Stopping/Reading: Carefully remove the Microplate sealing film, add 50 µl of Stop solution to each well, and mix gently to read the value. If wavelength correction is available, the wavelength can be set to 630 nm, and the reading at 630 nm can be subtracted from the reading at 450 nm (correcting optical defects in the plate). If only the OD value at a single wavelength of 450 nm is detected, a blank well should be set as a control, the blank well reading value should be deducted during calculation, and the reading directly at 450 nm without correction may be inaccurate.

07-3/Procedures for Simple Operation



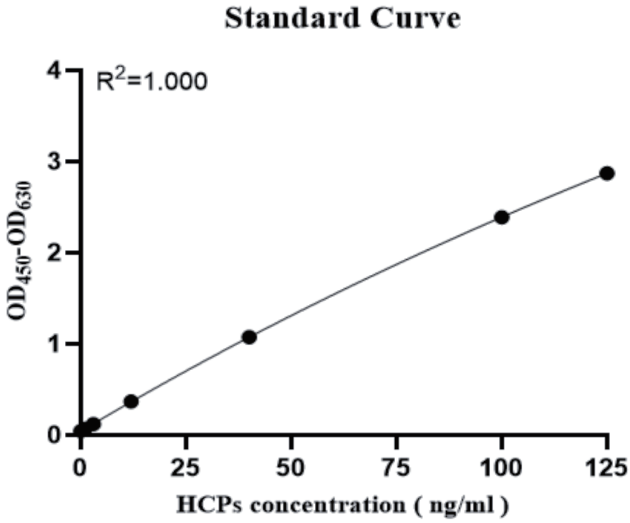
07-4/Quality Control

1. During quantitative detection, the correlation coefficient R^2 of the calibration curve should be ≥0.99, otherwise the experiment may be invalid.
2. During the experiment, it is recommended to include your company's or our company's quality control (QC) samples to ensure the validity of the experiment. Our company's QC samples recommends using the quality control components in the reagent kit.

08/Result Calculation

1. Calculate the mean optical density (OD) value of the sample replicates after subtracting the background absorbance (dual-wavelength correction). Plot the calibrator concentrations (ng/ml) on the X-axis and the OD values on the Y-axis. A four-parameter logistic (4PL) curve fit is recommended to generate the calibration curve. Interpolate the sample OD values into the calibrator curve equation to determine the sample concentration. Multiply by the appropriate dilution factor to calculate the actual concentration of the samples, which represents the HCPs concentration in each test sample.

2. If the sample OD value exceeds that of the Standard 1, the result is extrapolated from the curve. For accurate results, dilute the sample and retest. Calculate the concentration by interpolating the OD value of sample well into the calibration curve and multiplying by the corresponding dilution factor.
3. This calibration curve is only for demonstration, and a new calibration curve will be generated during each experiment.



09/Product Performance Indexes

1. Linearity and calibrator range: 1 - 125 ng/ml, with a linear correlation coefficient $R^2 \geq 0.99$.
2. Lower Limit of Quantification (LLOQ): 1.0 ng/ml.
3. Precision: The coefficient of variation is not greater than 15%.
4. Specificity: There is no cross reactivity with Vero, HEK293T, SF9, *E.coli* host proteins, and Pichia pastoris host proteins.

10/Limitations

1. This kit is specifically designed for the detection of host cell protein residues derived from CHO cell line production processes.
2. High or low pH values, detergents, urea, high salt concentrations, and organic solvents in the sample matrix are known interfering factors that may cause abnormal measurements. It is recommended to select an appropriate dilution factor before testing.
3. Because strong acid, strong alkali, strong oxidizing and reducing substances may change the enzyme activity, the operator should try to avoid the influence of these extreme environments in the whole process of detection.

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