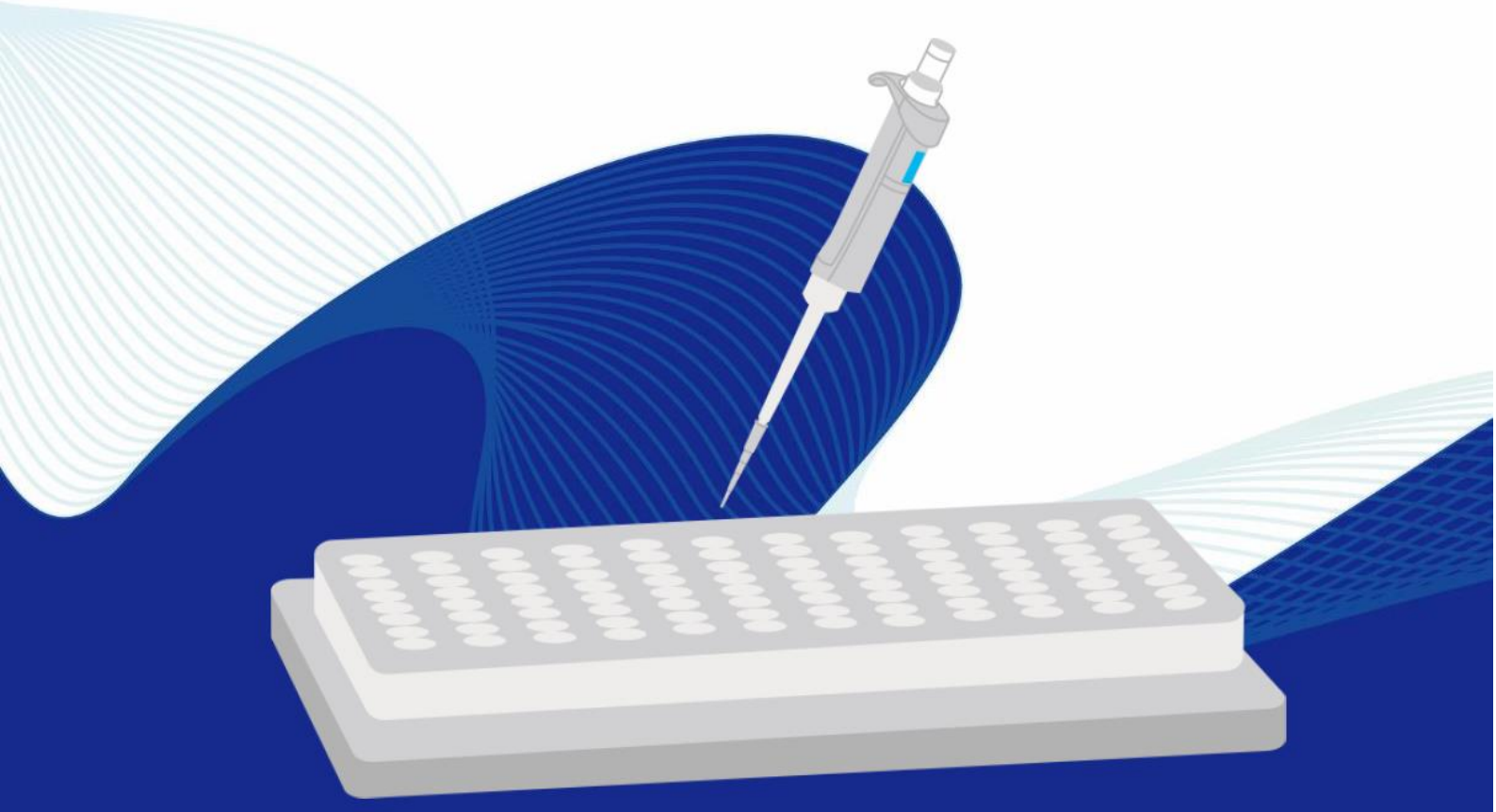
CHO HCP ELISA KIT（ready-to-use）

Product Manual



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1.1. Product Introduction

CHO cell line is a common and frequently used cell line in the commercial manufacturing of drug substance. During production and purification, host cell proteins (HCP) residues of CHO cells will reduce the efficacy of the drug product and cause toxicity and immune responses. Therefore, HCP impurities need to be reduced to the lowest level. Enzyme-linked immunosorbent assay (ELISA) is a widely accepted method to detect HCP residue levels. Compared with Western blot and other methods, ELISA method is more sensitive and easier to operate.

Anti-CHOk1 HCP antibody is produced in goats and purified by affinity chromatography. The specific antibody production process ensures that more than 80% of HCP can be detected, including both low and high molecular weight species. Therefore, this ELISA kit can be used to monitor HCP removal during process development, as well as for QC product release. 2D/WB method was used to characterize the coverage rate of anti-CHOK1 HCP antibody against CHOK1 HCP standard in the kit. The results showed that a high coverage rate of 80% was obtained. This kit is versatile because it can react specifically with CHOK1 HCP, independent of the purification process. Therefore, it can be used for early or late process development and quality control of most CHOK1 expression systems. However, if the user finds that the antibodies in the kit do not have good HCP coverage, a process-specific HCP detection kit needs to be developed.

The kit is used to detect the presence of host cell protein impurities in products expressed by CHO cell lines. The kit is intended for research and production use only and is not intended for diagnosis purpose in humans or animals.

1.2. Product Specification

- Detection method: colorimetric method
- Detection type: sandwich ELISA
- Sensitivity: 1 ng/mL
- Linear range: 3 ng/ ml - 100 ng/mL
- Estimated test time: 2.5 hours.

1.3. Operation Procedures

This ELISA kit uses one-step enzyme-linked immunosorbent assay (ELISA) method. Samples containing CHOK1 HCP can react with goat anti-CHOK1 antibodies labeled by HRP and anti-CHOK1 antibodies coated on well plate simultaneously. Finally, coated antibody – HCP - labeled antibody sandwich-like complex is formed. By washing the plate, unbound antigens and antibodies can be removed. After the TMB substrate was added into the well and fully reacted, the color development was stopped by adding the stop solution. The OD or light absorption value of the reaction solution at 450/650 nm was analyzed by the microplate reader. The OD value or light absorption value is proportional to the HCP content in the solution. Therefore, the concentration of HCP in solution can be calculated according to the standard curve.

Reagent and Material

Item	Description	Product Number	Concentration	Specification	Storage Conditions
1	CHOK1 HCP standard 1	DNPS030401-1	100 ng/mL	1 mL/Tube	2-8 ℃
2	CHOK1 HCP standard 2	DNPS030401-2	75 ng/mL	1 mL/Tube	2-8 ℃
3	CHOK1 HCP standard 3	DNPS030401-3	40 ng/mL	1 mL/Tube	2-8 ℃
4	CHOK1 HCP standard 4	DNPS030401-4	12 ng/mL	1 mL/Tube	2-8 ℃
5	CHOK1 HCP standard 5	DNPS030401-5	6 ng/mL	1 mL/Tube	2-8 ℃
6	CHOK1 HCP standard 6	DNPS030401-6	3 ng/mL	1 mL/Tube	2-8 ℃
7	Blank control	DNPS030401-7	0 ng/mL	1 mL/Tube	2-8 ℃
8	Anti-CHO HCP-HRP detection solution	DNPS030402-1	2 μg/mL	15 mL/Tube	2-8 ℃, keep in dark place
9	TMB	DNPS030403	NA	12 mL/Bottle	2-8 ℃, keep in dark place
10	20 * PBST 0.05%	DNPS030404	NA	15 mL/Bottle	2-8 ℃
11	Stop solution	DNPS030405	NA	12 mL/Bottle	RT
12	Sealing film for well plate	6050185	NA	3 pieces	RT
13	High adsorption pre-coated 96-well plate	DNPS030407	NA	1 plate (8 x 12 strips)	2-8 ℃
14	Dilution solution	DNPS030408	NA	50 mL/Bottle	2-8 ℃

Table 1 Main Reagents and Consumables in the Kit

Required equipment and materials (not provided in this kit)

Consumables/Equipment	Recommended supplier	Product number
Microplate reader	Molecular Devices	Spectra Max M5, M5e, or equivalent equipment
Thermostatic mixer	Eppendorf	Eppendorf/5355, or equivalent equipment
Vortex mixer	IKA	MS3 Digital, or equivalent equipment

Table 2 Required equipment and materials which are not provided in this kit.

Safety Precautions

The stop solution is 2M sulfuric acid, please handle it carefully to avoid splashing.

Reagent preparation

1. PBST 0.05 %

15mL 20*PBST 0.05%, diluted in ddH_20 to 300 mL.

Operation procedure

Equilibrate all reagents to room temperature.

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Add 50 µL of standards 1-6, samples and QC (see Table 3) into the wells, and then add 100 µL of detection solution (2 µg/mL). Seal the well plate with sealing film, place the well plate on the mixer, and incubate at 500 rpm and 25±3°C for 2 h.

↓

Place the plate upside down over the sink and discard the incubation liquid. Add 300 µL of PBST 0.05% into the wells, wash the wells and discard the solution, and repeat washing three times. Turn the plate upside down on a clean tissue and pat it dry.

→

Add 100 µL TMB substrate to each well (see Table 1), seal the plate, and incubate at 25±3°C in the dark for 15 minutes.

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Add 100 µL stop solution to each well (see Table 1).

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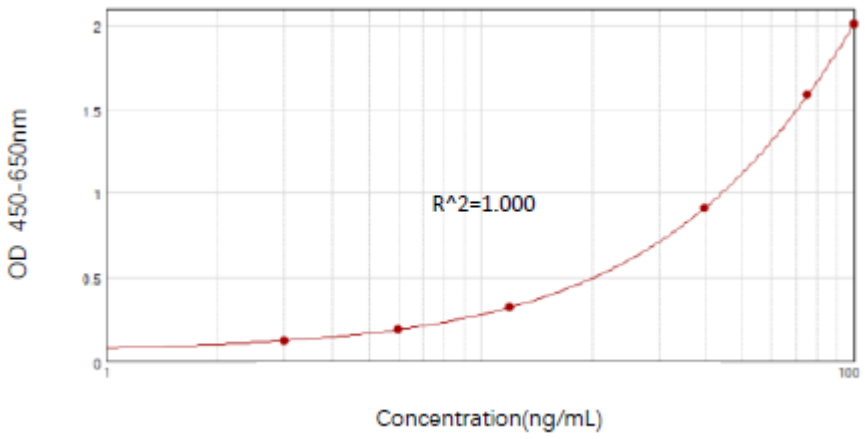
Analyze data using SoftMax or similar software. And use four-parameter fitting regression model to generate standard curve.

Tube number	Stock solution	Concentration (ng/mL)	Volume (µL)	Dilution solution volume (µL)	Total volume (µL)	Final concentration (ng/mL)
QC	Standard 1	100	50	200	250	20

Table 3 Preparation of QC

Standard curve example



Note

1. If the concentration of HCP in the sample exceeds the upper limit of the standard curve, the sample needs to be appropriately diluted with dilution buffer before testing.
2. To avoid getting bad CVs in the experiment, perform duplicate well operations.
3. It is recommended to include the 0 point of the standard curve in the fitting.
4. It is recommended that the pre-coated well plate should be used within a week after being unpacked.

Technical Support

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