# Instructions for Use of CHO HCP ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

**Cat. No. HG-HCP003**

## Introduction

BlueKit series CHO HCP ELISA Detection Kit is a specialized kit for the quantitative detection of residual CHO cell-derived host proteins.

This kit uses a double-antibody sandwich method. Add the gradient-diluted CHO-K1-HCP standard and the sample to be tested into the microtiter plate pre-coated with anti-CHO-K1-HCP antibody, then add the diluted biotin-labeled CHO-K1 HCP detection antibody, and finally add the diluted Streptavidin-HRP to form the antibody-antigen-antibody complex. Wash the plate and add TMB chromogenic reagent for color reaction. The concentration of CHO-K1-HCP is positively correlated with the color intensity.

Assay range: 1.56-100 ng/mL

Limit of quantification: 1.56 ng/mL

## Specification

96 T

## Usage

It is applicable to the optimization of purification process of biological products, impurity control of intermediate process and release testing of final products.

## Kit components

| Components | Specification | Preparation |
|---|---|---|
| CHO-K1 HCP Standard | 100μL | Gradient dilution with diluent Buffer 1. |
| Coated Plate | 8 wells × 12 strips | Ready-to-use |
| Dilution Buffer 1 | 50 mL x 1 vial | Ready-to-use |
| Dilution Buffer 2 | 30 mL x 1 vial | Ready-to-use |
| Wash Buffer (20×) | 50 mL × 1 vial | Make a 20-fold dilution with ultrapure water. |
| Detection Antibody (50×) | 300 μL × 1 tube | Make a 50-fold dilution with diluent buffer 2.. |
| Streptavidin-HRP (100x) | 300 μL × 1 tube | Make a 100-fold dilution with diluent buffer 2. |
| TMB Substrate | 12 mL × 1 vial | Ready-to-use |
| Stop Solution | 10 mL × 1 vial | Ready-to-use |
| Sealing Film | 5 pieces | Ready-to-use |
| Instructions for Use | 1 copy | Ready-to-use |

## Storage and shelf life

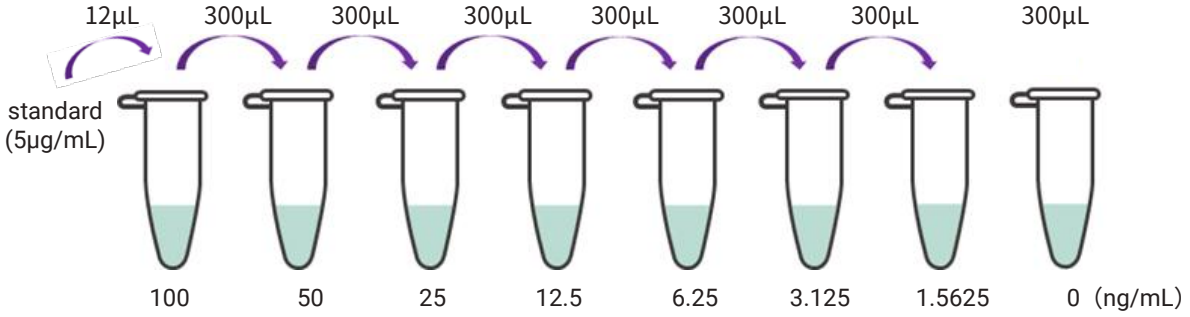
Sealed kits are valid for 12 months at 2-8°C .

## Apparatus and materials to be prepared by the user:

- Plate reader
- Thermostat plate shaker
- Micro pipette and tips
- Deionized waters
- Unused filter paper
- Vortex shaker

## Pre-experiment preparation

1. All reagents and samples to be tested should be restored to room temperature. All reagents are newly prepared and ready-to-use.
2. Preparation of 1x washing solution: Equilibrate the concentrated washing solution to room temperature, without crystallization. After mixing well, according to the usage volume, dilute 20x washing solution by 20 times with an appropriate amount of ultrapure water at the ratio of 1:19, to obtain 1x washing solution.
3. Preparation of 1x detection antibody: After the 50x detection antibody is fully dissolved, centrifuge and dilute the solution with Diluent 2 at the ratio of 1:49.
4. Preparation of 1x enzyme conjugate: After 100x enzyme conjugate is fully dissolved, centrifuge and dilute the solution with Diluent 2 at the ratio of 1:99.
5. Preparation of standards: Prepare eight 1.5 mL centrifuge tubes and label them in turn according to the concentrations of the standards. Add 12 microliters of standard substance and 588μL diluent 1 into the first centrifuge tube, and mix well to form ST1(100ng/mL). Add 300μL diluent 1 into the remaining 7 centrifuge tubes, and then perform gradient dilution according to the following figure.




## Operation procedures

1. Washing the coated plate: Wash the plate three times with 1x washing solution (300 μL/well) and pat dry.
2. Incubation of samples: Add standard and test sample correspondingly, 100 μL/well, and incubate at 37°C for 1 h.
3. Washing the coated plate: Discard the liquid in the wells, add 1x washing solution (300 μL/well), wash the plate for 5 times, and pat dry.
4. Incubation of detection antibody: Add the prepared 1x detection antibody into the wells, 100 μL/well, and incubate at 37°C for 1 h.
5. Washing the coated plate: Discard the liquid in the wells, add 1x washing solution (300 μL/well), wash the plate for 5 times, and pat dry.
6. Incubation of enzyme conjugate: Add the prepared 1x enzyme conjugate into the wells, 100 μL/well, and incubate at 37°C for 40 min.

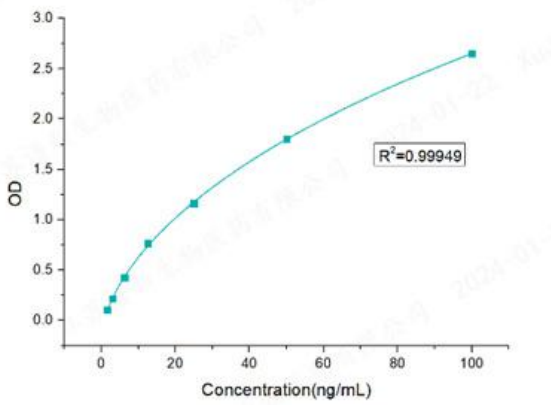
7. Washing the microtiter plate: Discard the liquid in the wells, add 1x washing solution (300 µL/well), wash the plate for 5 times, and pat dry.
8. Color development: Restore the chromogenic reagent to room temperature 10 min before use, add 100 µL to each well, and incubate at 37°C for 15 min in the dark.
9. Termination: Add 50 µL stop solution into each well, and gently shake the coated plate until the color is uniform.
10. Loading for testing: Load samples into the microplate reader in 10 min and read the absorbance value at 450 nm/630 nm (450 nm is the detection wavelength, 630 nm is the reference wavelength) on the microplate reader.

## Results process

1. OD processing of the standard curve (See the following example, which is only for example purpose. Please refer to the actual measurement for details):

| Standard concentration (ng/mL) | OD value (1) | OD value (2) | Mean value |
|---|---|---|---|
| 0.00 | 0.1229 | 0.1271 | 0.1250 |
| 1.5625 | 0.2268 | 0.2322 | 0.2295 |
| 3.125 | 0.3405 | 0.3385 | 0.3395 |
| 6.25 | 0.5444 | 0.5538 | 0.5491 |
| 12.5 | 0.8784 | 0.8985 | 0.8885 |
| 25 | 1.2892 | 1.2889 | 1.2891 |
| 50 | 1.9245 | 1.924 | 1.9243 |
| 100 | 2.7547 | 2.7934 | 2.7741 |

2. The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below).



## Precautions

1. When the sample is tested for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve.
2. The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.
3. Before using the coated microtiter plates, please equilibrate to room temperature and then open the secondary packaging. The strip plates not used in the test should be immediately placed back into the package and sealed properly, and can be stored at 4°C for one month. Other unused reagents should be packaged or covered properly.
4. Please use disposable tips during experimental operation to avoid cross contamination.
5. Please check each individual reagent in the kit fully before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.
6. When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well for liquid absorption.
7. The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.
8. The kit is intended for single use. Please use within the shelf life.

## Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.



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