# 酶联免疫法测定CHOK1宿主细胞蛋白

## 简介

CHO 细胞是一种常见的，并在商业大批量生产原料药经常使用的细胞系。在生产和纯化过程中，CHO 细胞产生的宿主细胞蛋白(HCP)会降低药物的疗效，并产生毒性和免疫反应。因此，需要将 HCP 杂质降低到最低水平。普遍受到大家认可的检测 HCP 水平的方法是酶联免疫法（ELISA），与 Western blot 等方法比，该方法灵敏度高，操作简便。

抗 CHOK1 HCP 抗体在山羊中产生，并用 CHOK1 HCP 亲和纯化。特异性的抗体产生流程确保 80% 以上的HCP，包括低分子量和高分子量的种类可以被检测到。因此，该试剂盒可以在工艺开发过程中用于监测 HCP 的移除，以及 QC 产品放行。采用 2D/WB 方法对试剂盒中抗 CHOK1 HCP 抗体对 CHOK1 HCP 标准品的覆盖度进行了表征，并且获得了 80% 的高覆盖率。这种试剂盒是通用的，因为它可以同 CHOK1 HCP 发生特异反应，而不依赖于纯化过程。因此可用于大多数 CHOK1 表达系统的早期或晚期的过程开发和质量控制。然而，如果用户发现试剂盒中的抗体没有较好的 HCP 覆盖度，则需要开发一种工艺特异性的 HCP 检测试剂盒。

该试剂盒用于测定CHOK1细胞系表达的产品中是否存在宿主细胞蛋白杂质。该试剂盒仅供研究和生产使用，不用于人类和动物的诊断。

## 产品参数

·检测方法：比色法
·检测类型：夹心ELISA
·灵敏度：1ng/ml
·线性范围：3ng/ml-100ng/ml
·预计检测用时：2.5小时

## 实验流程

本方法采用一步酶联免疫 ELISA 法。含有 CHOK1 HCP 的样品可以与 HRP 标记的山羊抗 CHOK1 抗体以及包被在酶标板上抗 CHOK1 抗体同时反应。最终形成一种夹心复合物的固相抗体 -HCP- 标记抗体。通过清洗酶标板可以去除未结合的抗原抗体。向孔中加入 TMB 底物充分反应后，加入终止液后停止显色，用酶标仪读取反应溶液在 450/650nm 处的 OD或吸光值。OD值或吸光值与溶液中的 HCP 含量成正比。因此，可以根据标准曲线计算出溶液中的 HCP浓度。

## 试剂与材料

| 序号 | 组成 | 货号 | 浓度 | 规格 | 储藏条件 |
|---|---|---|---|---|---|
| 1 | CHOK1 HCP 标准品 | DNPS030401 | 0.5 mg/mL | 70ul/管 | ≤–20°C |
| 2 | Anti-CHO HCP-HRP | DNPS030402 | 0.5 mg/mL | 60ul/管 | ≤–20°C, 避光 |
| 3 | TMB | DNPS030403 | NA | 12ml/瓶 | 2-8°C, 避光 |
| 4 | 20*PBST0.05% | DNPS030404 | NA | 15ml/瓶 | 2-8°C |
| 5 | 终止液 | DNPS030405 | NA | 12ml/瓶 | RT |
| 6 | 酶标板封口膜 | 6050185 | NA | 3张 | RT |
| 7 | BSA | DNPS030406 | NA | 1g | 2-8°C |
| 8 | 高吸附 96 孔预包酶标板 | DNPS030407 | NA | 1块 | 2-8°C |

表 1 试剂盒中主要试剂和耗材



## 需要但是未提供的设备和材料

| 耗材/设备 | 厂家 | 货号 |
|---|---|---|
| 酶标仪 | Molecular Devices | Spectra Max M5，M5e，或等效设备 |
| 恒温混匀仪 | Eppendorf | Eppendorf/5355 ，或等效设备 |
| 漩涡混匀仪 | IKA | MS3 Digital，或等效设备 |

表 2 需要但是未提供的设备

## 安全措施

终止液是 2M 硫酸，请小心处理，防止飞溅。

## 试剂制备

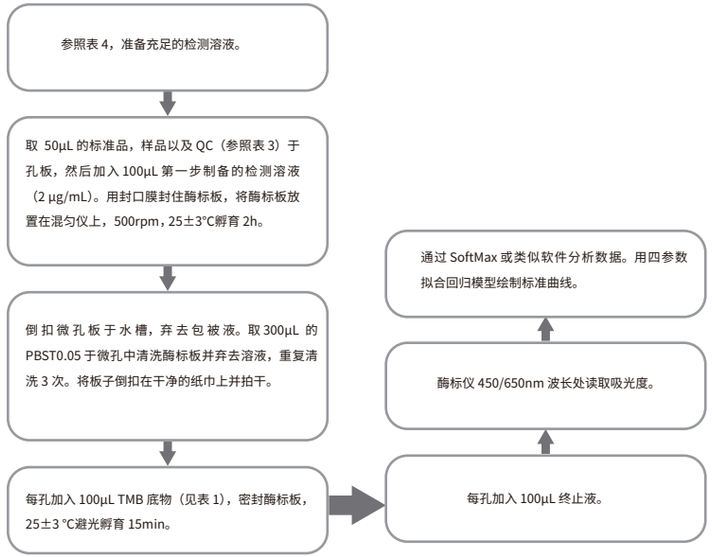
1. PBST0.05%

15ml 20*PBST 0.05%，稀释于 $ddH_2O$ 中，定容至 300ml。

2. 1.0% BSA

将瓶中的 1g BSA 溶于 100ml PBST0.05% 中，充分混匀至完全溶解，2-8°C储存，制备好的稀释缓冲液有效期为 7 天 , 建议按需配制。

## 实验流程





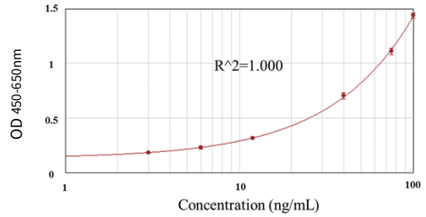

| 管号 | 原液 | 浓度(ng/mL) | 体积(μL) | 1%BSA 体积 (μL) | 总体积(μL) | 终浓度(ng/mL) |
|---|---|---|---|---|---|---|
| A | CHOK1 HCP 标准品 | 0.5 mg/mL | 10 | 490 | 500 | 10000 |
| B | A | 10000 | 50 | 450 | 500 | 1000 |
| S1 | B | 1000 | 50 | 450 | 500 | 100 |
| S2 | S1 | 100 | 300 | 100 | 400 | 75 |
| S3 | S2 | 75 | 200 | 175 | 375 | 40 |
| S4 | S3 | 40 | 150 | 350 | 500 | 12 |
| S5 | S4 | 12 | 200 | 200 | 400 | 6 |
| S6 | S5 | 6 | 200 | 200 | 400 | 3 |
| NC | NA | NA | NA | 200 | 200 | 0 |
| QC | S1 | 100 | 50 | 200 | 250 | 20 |

表 3 QC 和标准品的配置

| 名称 | Anti-CHO HCP-HRP 体积 | 1%BSA | 终浓度 |
|---|---|---|---|
| 检测液 | 48 μL of 0.5 mg/mL | 11952 μL | 2 μg/mL |

表 4 检测溶液的制备

## 标准曲线示例




## 备注

1. 如果样品中 HCP 的浓度超过标曲的上限，则样品需要在测试前用稀释缓冲液适当稀释。
2. 为避免试验中不好的 CVs，请进行复孔操作。

## 技术支持

有任何技术问题或技术支持，请联系：marketing@duoningbio.com

# Immunoenzymetric Assay for the Measurement of CHOK1 Host Cell Proteins

## Introduction

CHO cells is a common used cell line for production of commercial quantities of a drug substance. Host cell proteins (HCPs) from CHO cells produced in manufacturing and purification process can reduce the efficacy of the therapeutic agent and result in adverse toxic or immunological reactions and thus it is desirable to reduce HCP impurities to the lowest levels. The microtiter plate based immunoenzymetric assay (ELISA) method are conventionally accepted to detect the levels of HCP. Compared with other methods, such as Western blot, this method has a better sensitivity and easy to operate.

The anti-CHOK1 HCP antibodies have been generated in goats and affinity-purified by using CHOK1 HCPs. Proprietarily procedures for the antibody generation ensure that over 80% of HCP components including both low molecular weight and high molecular weight species can be detected by the antibodies. Therefore, this kit can be used as a process development tool to monitor the optimal removal of host cell impurities, as well as a QC product release assay. Characterization of coverage of the anti-CHOK1 HCP antibodies in the kit to the CHOK1 HCP standards was performed by using 2D Gel/2D Western blot method, and a high coverage of 80% was obtained.

This kit is "generic" in the sense that it is intended to react with essentially all of the HCPs that could contaminate the product independent of the purification process. Thus it can be used in most CHOK1 based expression system in early or late phase process development and quality control. However, if the antibody in the kit was found having insufficient HCP coverage, a process-specific HCP detection kit has to be developed.

This kit is intended for use in determining the presence of host cell protein impurities in products manufac- tured by expression in the CHOK cell line. The kit is for Research and Manufacturing use only and is not intended for diagnostic use in humans or animals.

## Product Parameters

- detection method：colorimetric method
- Assay type：sandwich ELISA
- Sensitivity：1ng/ml
- Range：3ng/ml-100ng/ml
- Estimated time of experiment：2.5h

## Experimental Protocol

A two-step immunoenzymetric ELISA method is used in this assay. Samples containing CHOK1 HCP are reacted simultaneously with a HRP conjugated goat anti-CHOK1 antibody in microplate coated with an affinity purified capture anti-CHOK1 antibody. The immunological reactions result in the formation of a sandwich complex of solid phase antibody-HRP-enzyme labeled antibody. The mocroplate is washed to remove any unbound materials. The TMB peroxide substrate solution is added to the wells. The color development is stopped, and the optical (OD) or absorbance of the reaction solution at 450 minus 650nm is read on a microplate reader. The OD or Absorbance is proportional to the amount of HCP in the solution. Thus the concentration of HCP in the solution can be calculated according to the standard curve.

### Reagents & Materials Provided

| S/N | Component | Item number | Concentration | specification | Storage Upon Receipt |
|---|---|---|---|---|---|
| 1 | CHOK1 HCP standard | DNPS030401 | 0.5 mg/mL | 70ul/vial | ≤-20°C |
| 2 | Anti-CHO HCP-HRP | DNPS030402 | 0.5 mg/ml | 60ul/vial | ≤-20°C, protect from light |
| 3 | TMB | DNPS030403 | NA | 12ml/bottle | 2-8°C, protect from light |
| 4 | 20*PBST0.05% | DNPS030404 | NA | 15ml/bottle | 2-8°C |
| 5 | Stop solution | DNPS030405 | NA | 12ml/bottle | RT |
| 6 | Microplate sealers | 6050185 | NA | 3 pics | RT |
| 7 | BSA | DNPS030406 | NA | 1g | 2-8°C |
| 8 | Pre-coating Plates | DNPS030407 | NA | 1pics | 2-8°C |

Table 1 Reagents and Materials provided in the kit



### Materials & Equipment Required But Not Provided

| Materials/Equipment | Manufacture | Catalog |
|---|---|---|
| Microplate Reader | Molecular Devices | Spectra Max M5, M5e or equivalent |
| Thermomixer | Eppendorf | Eppendorf/5355 or equivalent |
| Vortex mixter | IKA | MS3 Digital or equivalent |

Table 2 Equipment required but not provided

### Safety precautionst

The stop solution is 2 M sulfuric acid, be careful and avoid splashing.

### Preparation of reagents

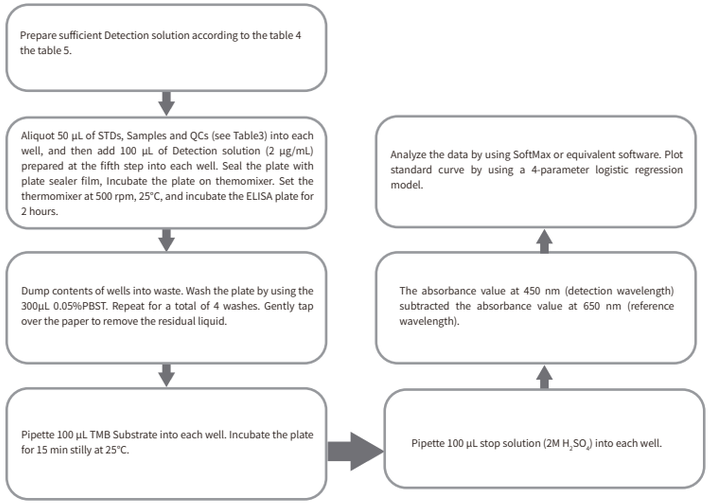
1. PBST0.05%

   15ml 20 * PBST 0.05%, diluted in dd$H_2O$, constant volume to 300ml.

2. 1.0% BSA

   Dissolve 1g BSA in 100ml PBST 0.05%, mix well until it is completely dissolved, store at 2-8 °C, and the validity of the prepared diluent buffer is 7 days, Adjust the serial dilution schemes accordingly.

### Assay Protocol





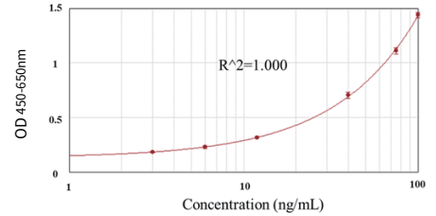

| Tube NO. | Original Solution | Concentration (ng/mL) | Volume (µL) | 1%BSA Volume(µL) | Total Volume (µL) | Final Concentration (ng/mL) |
|---|---|---|---|---|---|---|
| A | CHOK1 HCP standard | 0.5 mg/mL | 10 | 490 | 500 | 10000 |
| B | A | 10000 | 50 | 450 | 500 | 1000 |
| S1 | B | 1000 | 50 | 450 | 500 | 100 |
| S2 | S1 | 100 | 300 | 100 | 400 | 75 |
| S3 | S2 | 75 | 200 | 175 | 375 | 40 |
| S4 | S3 | 40 | 150 | 350 | 500 | 12 |
| S5 | S4 | 12 | 200 | 200 | 400 | 6 |
| S6 | S5 | 6 | 200 | 200 | 400 | 3 |
| NC | NA | NA | NA | 200 | 200 | 0 |
| QC | S1 | 100 | 50 | 200 | 250 | 20 |

Table 3 An example for Preparation of Standards and QC

| Name | Anti-CHO HCP-HRP Conjugate Volume | 1%BSA | Final Concentration |
|---|---|---|---|
| Detection solution | 48 µL of 0.5mg/mL | 11952µL | 2 µg/mL |

Table 4 An example for Preparation of Detection solution

### Example Standard Curve




### Notest

1、If the HCP content in the sample is more than the highest point of standard, the sample should be diluted with the Dilution buffer appropriately before the test.

2、Operate parallel as soon as possible to avoid the poor CVs during the experiment.

### Technical Support

Please contact Duoning Biology for any technical questions and supports at: marketing@duoningbio.com