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

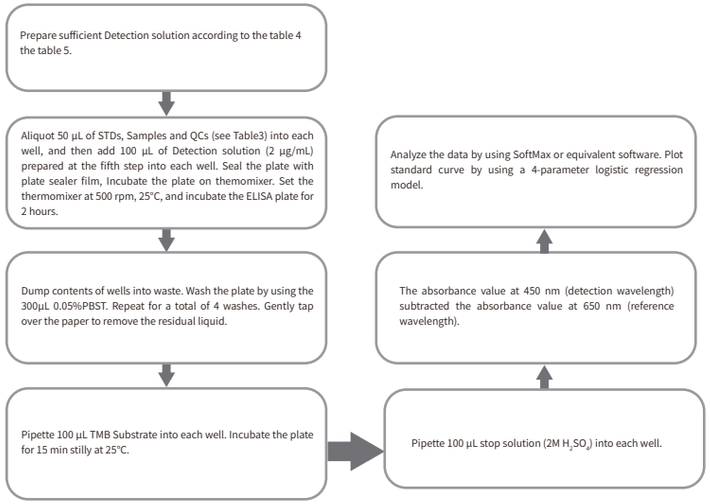
1. PBST0.05%

   15ml 20 * PBST 0.05%, diluted in ddH_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



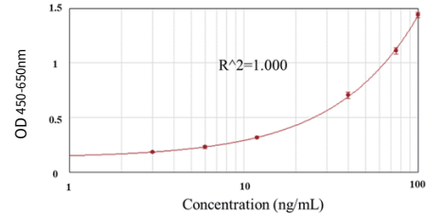
Tube NO.	Original Solution	Concentration (ng/mL)	Volume (µL)	1%BSA Volume(µL)	Total Volume (µL)	Final Concentration (ng/mL)
A	CHOK1 HCP standard	0.5 mg/mL	10	490	500	10000
B	A	10000	50	450	500	1000
S1	B	1000	50	450	500	100
S2	S1	100	300	100	400	75
S3	S2	75	200	175	375	40
S4	S3	40	150	350	500	12
S5	S4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