

Chinese Hamster Ovary Host Cell Proteins

Catalog. No: KBBP03

Immunoassay for the measurement of CHO Host Cell Proteins

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BIOSYSTEMS is strictly prohibited.

KRISHGEN BIOSYSTEMS 135/37, Sonawala Bldg., 2nd Floor, Zaveri Bazar, Mumbai 400 002
Tel: 91-22-66372990/91 Fax: 91-22-66372991 E-mail: sales@krishgen.com Website: www.krishgen.com

Introduction:

A variety of proteins which are used as therapeutic agents in humans and animals are produced through recombinant expression in *Chinese Hamster Ovary (CHO) cells*. The manufacturing and purification process of these products tends to leave the potential for contamination by Host Cell Proteins (HCPs) from CHO cells, which may result in adverse toxic or immunological reactions that ultimately affect the efficacy of the therapeutic agent. The simple, objective and semi-quantitative ELISA is a highly sensitive method that aids in purification process development, process control, quality control and product release testing optimally.

Intended Use:

This generic kit is intended in determining the presence of *Chinese Hamster Ovary* Host Cell Protein contamination in various products that are manufactured through recombinant expression in CHO cells. The kit has been validated successfully for testing of final product HCPs in variety of products regardless of growth and purification process.

Principle:

This assay is based on the Sandwich ELISA procedure. Samples containing CHO HCPs are reacted with anti-CHO:HRP antibody simultaneously in the microtiter wells already coated with affinity purified capture anti-CHO antibody. This immunological reaction results in formation of a sandwich complex of solid phase antibody-HCP-enzyme labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure (see Assay procedure section mentioned below). The substrate TMB is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and it is directly proportional to the concentration of CHO HCPs present.

Materials Provided:

1. Anti-CHO coated Microtiter plate (96 wells) – 1 no
2. Anti-CHO:HRP Conjugate, 11mL – 1 bottle
3. CHO HCP Standards, (0.5mL/vial) – 0, 1, 4, 20, 75 & 250 ng/mL
4. 3,3',5,5' Tetramethyl benzidine Substrate, 12mL – 1 bottle
5. Wash Buffer (10X), 100 mL – 1 bottle
6. Stop Solution, 12mL – 1 bottle
7. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm and 630 nm
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 μ L to 100 μ L
3. Distilled water
4. Wash bottle or automated microplate washer
5. Log-Log graph paper or software for data analysis
6. Timer
7. Absorbent paper
8. 1 Liter bottle for diluted Wash buffer

Handling/Storage:

1. All reagents should be stored at 2^oC to 8^oC for stability.
2. All the reagents and wash solutions are stable until the expiration date of the kit.
3. Before using, bring all components to Room temperature (18-25 °C). Upon assay completion return all components to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research or Manufacturing use only.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Dilute Wash Buffer to 1 liter in distilled water and store at 4^oC.
3. Bring all reagents to Room temperature before use.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of HCP. High Dose Hook Effect is due to insufficient excess of antibody for very high concentrations of HCPs present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the HCP concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN₃), as it could destroy the HRP activity of the conjugate resulting in under-estimation of the amount of CHO Host Cell Proteins.
4. All Standards, Controls and Samples should be assayed atleast in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Standards, Controls and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. A standard curve is required for each assay.
2. Pipette out 25 μ L of **Standards, Controls** and **Samples** into the respective wells as mentioned in the worklist.
3. Pipette out 100 μ L of anti-CHO:HRP **Conjugate** into each well.
4. Cover the plate and incubate it on a plate shaker at ~180rpm for 90 minutes at room temperature, $22^{\circ}\text{C} \pm 4^{\circ}$ or incubate for about 120 minutes without shaking.
5. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
6. Pipette out 100 μ L of TMB **Substrate** in each well.
7. Incubate the plate at room temperature for 30 minutes. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
8. Pipette out 100 μ L of **Stop Solution**. Wells should turn from blue to yellow in color.
9. Read the absorbance at 450/630 nm blanking on the Zero Standard.

Example of a Work list

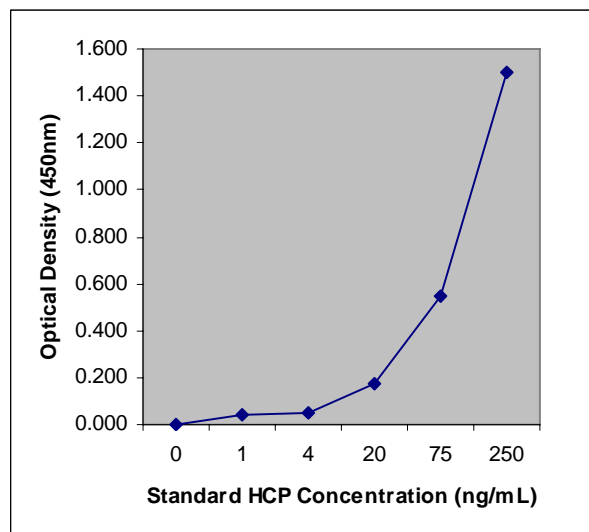
Well #	Contents	Abs at 450/630nm	Mean Absorbance	ng/ml HCP equivalents
1A	Zero Std			
1B	Zero Std			
1C	1 ng/mL			
1D	1 ng/mL			
1E	4 ng/mL			
1F	4 ng/mL			
1G	20 ng/mL			
1H	20 ng/mL			
2A	75 ng/mL			
2B	75 ng/mL			
2C	250 ng/mL			
2D	250 ng/mL			
2E	Sample A			
2F	Sample A			
2G	Sample B			
2H	Sample B			

Calculation of Results:

Determine the Mean Absorbance for each set of duplicate or triplicate Standards, Controls and Samples. Subtract the Mean Absorbance of Zero Standard (background) from each well. Plot the Standard curve on log-log graph paper, with Standard HCP concentration on X-axis and Absorbance on Y-axis. Draw the best fit straight line through the Standard points. To determine the unknown HCP concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the Standard curve. At the point of intersection, draw a vertical line to the X-axis and read the HCP concentration. If the samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.



Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

THANK YOU FOR USING KRISHGEN PRODUCT !

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems. 2009

Performance Specifications

Sensitivity

The lower limit of detection (**LOD**) is defined as the lowest concentration of the analyte that can be distinguished from the absence of that analyte within a stated confidence limit. LOD for this assay is 1.65 ng/ml.

The lower limit of quantitation (**LOQ**) is defined as lowest level of analyte that can be reliably measured with acceptable accuracy and precision. LOQ for this assay is ~4 ng/ml.

Precision

Intra (n=10 replicates) and inter-assay (n=5 assays) precision were determined in duplicates with the low (~1.0 ng/ml) and high standard (~250ng/ml).

	Intra Assay	Inter Assay
	CV %	CV %
Low	5.4%	7.9%
High	4.8%	5.0%

Hook Capacity

Increased concentrations of CHO HCP >250 ng/ml were assayed as unknowns. The hook capacity was ~125 ug/ml.

Recovery Estimation/ Interference Studies

Known amounts of CHO HCPs were added with different buffer matrices used to make the standards in this kit. The reactivity of the assay is subject to running the assays by the operator, pipetting & washing techniques, incubation time or temperature, composition of reagents and the shelf life of the kit. Hence adjustments may be required to position the standard curve and/or samples in the desired range. pH changes as well as some detergents like SDS and Tween, sodium azide can cause under-estimation. Very high concentrations of certain proteins can also interfere in accurate detection of CHO HCPs. It is recommend that every user should validate their sample matrices. This may be done by diluting one part of the 250ng/mL standard provided with this kit into 4 parts of the sample matrix in question. Recovery should be to the order of 45 to 60 ng/ml CHO HCP.

Specificity/Cross-Reactivity

Extensive studies on cross-reactive have not been performed. However the antibodies used to manufacture this kit are specific for CHO HCPs. An analysis by immunoblot against other strains of CHO cells show that most of the proteins are conserved among all strains. Hence the assay can be used for detecting the majority of HCPs from all CHO cell lines.