VALIDATION OF KRIBIOLISA High Five HCP ELISA (Catalog No: KBBP21) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical ProceduresGuideline recommended by FDA (guidelines May 2022).

Document History

First Codification	History	Date
Version#1	**High Five HCP ELISA (Catalog No: KBBP21)**	30th June 2024

Approved: Quality Control	Approved: Product Development
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Introduction

This document presents a discussion of the characteristics of Krishgen's KRIBIOLISA High Five HCP ELISA (Catalog No: KBBP21). This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays. The document is prepared based on tests run in our laboratory and does not necessarily seek to cover the testing that may be required at user's end for registration in, or regulatory submissions. The objective of this validation is to demonstrate that it is suitable for its intended purpose - quantitative determination of High Five HCP contaminants in biopharmaceutical products.

Validation characteristics considered by us in accordance with the guidelines are listed below:

- **Sensitivity**
- **Specificity**
- **Precision**
- **Matrix Interference / Hook Effect**
- **Stability**

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at the minimum; the spike and recovery assay to assure quality results. For a more comprehensive validation, the user may run the protocols as suggested by us herein below to develop the parameters for quality control to be used with the kit.

For any queries or support on the data and its performance, please contact us at sales1@krishgen.com.

Background

H5 cell (BTI-Tn-5B1-4) is a clone isolated from ovary cells of female Trichoplusia ni insect, which is used to express recombinant proteins using Baculovirus expression vector system. This host cell is adapted to grow in serum-free medium. Ten-fold higher recombinant protein expression in H5 cells compared to SF9 cells has attracted the attention of pharmaceutical companies to produce recombinant protein products in this cell line. In this context, Cervarix, a FDA approved HPV vaccine, has been manufactured in Trichoplusia ni insect cell line. The products manufactured in this system have the potential of impurity with host cell proteins (HCPs). These impurities can affect the efficacy of the vaccine and lead to adverse toxic or allergic reactions. Therefore, it is desirable to minimize HCP impurities in the recombinant biopharmaceutical products.

Validation Information

1. Sensitivity:

The lower limit of detection (LOD) is defined as that concentration corresponding to a signal three standard deviations above the mean of the zero standard. LOD is ~0.8 ng/mL. The limit of quantitation (LOQ) is the lowest concentration at which CV is less than 20% with acceptable accuracy. The LOQ is ~3 ng/mL.

2. Specificity:

The H5 HCP ELISA kit can specifically measure the amount of HCP of the H5 host cell and does not interfere with the proteins of other expression hosts, including E. coli and CHO. It can also detect H5 HCP with different concentrations of HCP in the drug samples in different stages of production.

3. Precision:

Both intra- (n=20 replicates) and inter- (n=20 replicates) assays precision was determined on three drug substance samples with different concentrations of H5 HCP. Results are shown in Tables 1 and 2:

Table 1. Intra-Assay

Sample	No. of tests* performed	Means (ng/mL)	SD (ng/mL)	CV (%)
1	20	10.56	0.36	3.4
2	20	39.97	1.38	3.45
3	20	92.23	9.02	9.78

Table 2. Inter-Assay

Sample	No. of tests* performed	Means (ng/mL)	SD (ng/mL)	CV (%)
1	20	10.74	0.82	7.64
2	20	40.66	2.23	5.48
3	20	82.34	7.85	9.53

**Each test has been run in duplicate.*

4. Matrix Interference:

By spiking H5 HCP antigen with a specified concentration of 30 ng/ mL in the pharmaceutical sample containing known amount of H5 HCP, interference related to other proteins was not observed. Also, by spiking H5 HCP antigen in the samples containing different concentrations of BSA from 1 to 8 mg, matrix interference was not observed. The recovery test results were in the range of 80-120%.

5. Hook Effect:

To rule out possible hook effect occurrence, the H5 HCP assay was done on samples with high concentration of H5 HCP (up to 750 µg/mL) and no “hook effect” was seen.

Schematic Procedure of H5 HCP test		
Reagent	Standard	Sample
Standard	100 µL	-
Sample	-	100 µL
Shake the plate gently for 15 seconds and then cover the wells with cardboard sealer. Incubate for 90 minutes on the rotator at 200 rpm at RT. Remove the cardboard sealer of the plate and empty the contents. Wash the wells 5 times according to the washing instructions.		
Anti- H5 HCP-HRP conjugate	100 µL	100 µL
Cover the wells with the cardboard sealer. Incubate for 30 minutes on a rotator at 200-600 rpm at RT. Remove the cardboard sealer from the plate and empty the contents of the wells. Wash the wells 5 times according to the washing instructions.		
TMB	100 µL	100 µL
Incubate wells for 15 minutes at RT in dark.		
Stop	100 µL	100 µL
Read absorbance at 450 nm (use 630 nm as reference filter if it is available).		

6. Stability

A standard accelerated stability test for a sealed kit was conducted at 37°C and 2-8°C to get relevant data. The kit performed well at elevated temperatures and was considered to have acceptable performance for upto 12 months from the date of manufacturing.

Elisa Kit (n=5)	37°C for 1 month	2-8°C for 6 months
Average Recovery	80%	95 - 100%

Typical Result Calculations

1. Calculate mean absorbance value of standards and samples at 450 nm (use 630 nm filter as reference filter if it is available).
2. Construct a 4-PL fit standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL using curve fitting software, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Calculate the corresponding concentration of H5 HCP in the sample (ng/mL) from the standard curve using the mean absorbance value for each sample.
4. Mean absorbance value for each sample.

Example of H5 HCP Standard Curve

Standards (ng/mL)	OD (450/630 nm)	Mean OD
0	0.17	0.16
	0.15	
3	0.31	0.32
	0.33	
6	0.49	0.48
	0.48	
12	0.73	0.75
	0.78	
25	1.20	1.19
	1.18	
50	1.66	1.67
	1.68	
100	2.44	2.42
	2.40