VALIDATION OF KRIBIOLISA™ HEK HCP ELISA (CATALOG NO. KBBP15) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA (guidelines May 2018)

Document History

First Codification	History	Date
Version#1	**VALIDATION DATA OF KRIBIOLISA™ HEK HCP ELISA** (Cat No #KBBP15)	05.05.2024

Approved Quality Control	Approved Product Development	Approved Operations Head
		
Dr Prairna B	Atul G	Dr Atahar H

Introduction

This document presents a discussion of the characteristics of our KRIBIOLISA™ HEK HCP ELISA considered by us during the validation of this kit in accordance with ICH Q2 (R1) guidelines. 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 - detection of HEK HCP.

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

- **Sensitivity**
- **Specificity / Cross reactivity**
- **Precision**
- **Accuracy**
- **Matrix Effect**
- **Antibody Coverage**
- **Robustness**
- **Validation kit specific details**
- **Comparison details**

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

1. Sensitivity:

Limit Of Quantification:

The lower limit of quantification (LLOQ) of the assay is 6 ng/mL, and the upper limit of quantification (ULOQ) is 540 ng/mL. The CV was less than 20% and the relative bias was within ±20%.

Conc. (ng/mL)	CV (%)	Relative bias (%)
6 (n=10)	3.31	1.9
540 (n=10)	3.0	-1.7

Limit of Detection:

The detection limit is defined as the detection concentration corresponding to the average value of the blank +2*SD, and the detection limit of the kit is less than 1 ng/ml.

2. Specificity / Cross Reactivity:

Specificity:
The HCPs of commonly used cell lines were prepared at 5 ug/ml in Standard Diluent and assayed for cross-reactivity. The HCPs of SF-9 and *P.pastoris* showed no cross-reactivity to the assay.

Host Cell Proteins	Spiked conc. (ng/ml)	Recovery Rate (%)
SF-9 HCP	15	117.8
	432	78.2
P.pastoris HCP	15	116.2
	432	85.5

3. Precision:

3.1 Repeatability:
Precision was determined by analyzing at least 10 replicates. The CV was less than 20%.

Pool	Intra Assay %CV
Low	3.11
Medium	3.81
High	3.04

3.2 Inter-Assay:

Two batches of the kit were tested in two separate assays to assess inter-assay precision. The CV was less than 25% for LLOQ and ULOQ samples, and less than 20% for all other samples.

QC	Sample (ULOQ) n=3		Sample (high) n=3		Sample (medium) n=3		Sample (low) n=3		Sample (LLOQ) n=3	
Batch	1	2	1	2	1	2	1	2	1	2
Conc (ng/ml)	540		432		216		15		6	
Mean Conc (ng/ml)	521.86	537.23	445.01	456.21	236.52	219.25	15.01	14.98	5.12	4.98
CV (%)	3.1		3.8		5.3		4.5		11.68	

4. Accuracy

The Quality Control samples (QCs) were prepared at 5 concentration levels within the calibration curve range: LLOQ (6 ng/ml), low QC (15 ng/ml), medium QC (216 ng/ml), high QC (432 ng/ml) and ULOQ (540 ng/ml). The recovery rate was 75% - 125% for LLOQ / ULOQ samples, and 80-120% for all other samples.

QCs	Sample (ULOQ) n=3	Sample(high) n=3	Sample (medium) n=3	Sample (low) n=3	Sample (LLOQ) n=3
Conc. (ng/ml)	540	432	216	15	6
Average. Value (ng/ml)	523.12	423	208	13.8	5.5
Recovery Rate (%)	96.87	97.92	96.30	92.00	91.67

5. Matrix Effect

The recovery rate of HEK293 HCP spiked to 6ng/mL (LLOQ) in commonly used matrices was evaluated. The tested matrices showed no interference to the assay.

Sample Matrix	Spiked Conc (ng/ml)	Recovery Rate (%)
1×PBS, 0.075% Tween-20, 0.5% BSA, pH 6.0	6	95.0
1×PBS, 0.075% Tween-20, 0.5% BSA, pH 8.5	6	73.3
Hanks' Balanced Salt Solution (HBSS) (2-fold dilution)	6	101.7

6. Antibody coverage

The HCP antibody coverage of HEK293 HCP ELISA Kit was evaluated by Immunomagnetic Bead Separation (IMBS) method combined with LC-MS (IMBS-LC/MS) analysis. The antibody coverage obtained by was well characterized.

7. Robustness

a. Incubation Condition

The assay is designed to conduct at 25°C±3ºC. The suitable speed for incubation is at 500 - 600 rpm. The CV was less than 20% and the relative bias was within ±20%.

Temperature	22ºC		25ºC		28ºC	
Incubation Speed	500 rpm		500 rpm		600 rpm	
	Sample (low)	Sample (high)	Sample (low)	Sample (high)	Sample (low)	Sample (high)
QCs	n=4	n=4	n=4	n=4	n=4	n=4
Theoretical Conc.						
(ng/ul)	15	432	15	432	15	432
Average Value (ng/ul)	16.58	432.91	16.81	432.34	16.54	438.41
CV(%)	4.7	5	3.9	5.7	9.6	4.5
Relative Bias (%)	10.6	0.2	12.1	0.1	10.3	1.5

8. Validation Kit Specific Details:

Conc (ng/ml)	Mean value (ng/ml)	CV(%)	Relative Bias (%)
6	5.35	89.24	-10.8
18	20.75	115.30	15.3
54	50.47	93.46	-6.5
135	131.38	97.32	-2.7
270	286.22	106.01	6.0
540	527.07	97.61	-2.4

9. Krishgen & Cygnus HEK 293 HCP Kit Comparison

Sample: Retroviral vector based CGT* product

Sample	Krishgen Kit		Cygnus Kit	Krishgen / Cygnus
	Value (ng/ml)	Recovery	Value (ng/ml)	
In-Process Sample 1	8182	/	1418	5.7
In-Process Sample 2	51456	/	3561	14.4
Post-Harvest Final QC Sample	1136	/	457	2.4

Sample: Lentiviral vector based CGT* product

Sample		Krishgen Kit	Cygnus Kit	Krishgen Detection Concentration / Cygnus Detection Concentration
		Concentration (ng/ml)	Concentration (ng/ml)	
1	1-1 Harvest	30617.2	18012.1	1.7
	1-2 In-process	9115.2	1437.7	6.3
	1-3 Final	1621.5	715.2	2.26
2	2-1 Harvest	26577.2	12119.2	2.1
	2-2 In-process	2495.6	902.2	2.7
	2-3 Final	1498.2	612.3	2.4

** CGT = cell gene therapy products*

Experiment Aim: Cygnus Make Standards from their HEK HCP kit were tested with Krishgen's HEK HCP ELISA

Sample	Labeled Conc. (ng/ml)	Reported Conc. (ng/ml)	Test vs Label
Cygnus Standards	50	161.27	3.22
	25	80.02	3.20
	25	81.15	3.24

Experiment Aim: Krishgen Make Standards from their HEK HCP kit were tested with Cygnus' HEK HCP ELISA

Sample	Labeled Conc. (ng/ml)	Reported Conc. (ng/ml)	Test vs Label
Krishgen Standards	54	6.98	0.129
	18	1.75	0.097

Conclusion:

There are differences in the composition of HCP standards across different commercial kits, leading to variations in antibody coverage and abundance, which leads to discrepancies in sample detection values.

Cross-validation results show that the KISHGEN HEK HCP ELISA produces higher-than-expected values when testing Cygnus standards, while Cygnus produces lower-than-expected values when testing KRISHGEN's standards.

When both kits tested the same samples, the KRISHGEN's kit results were closer to the theoretically estimated concentration compared to Cygnus, indicating that KRISHGEN's antibodies provide higher coverage and recognition of the samples than Cygnus.

Disclaimer:
Please note that the Cygnus kit is sourced as a competitor and we have tested using a particular lot only. All comparisons are done on the basis of data generated using that particular kit only and we do not claim or offer any conclusions on the kits produced by them.

References:

- *USP <1132> Residual Host Cell Protein Measurement in Biopharmaceuticals*
- *EP <2.6.34> HOST-CELL PROTEIN ASSAYS*
- *ICH Q2 (R2) VALIDATION OF ANALYTICAL PROCEDURES*
- *ICH M10 on bioanalytical method validation*
- *ChP <9012> Guidance for method validation of quantitative analysis of biological samples*
- *Chinese pharmaceutical industry standard: YY/T1183-2010 Elisa reagent (kit)*

Validation Guide Ver1.0 dated 05.05.2024