VALIDATION OF KRIBIOLISA™ HEK HCP ELISA AS PER ICH M10 GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA (guidelines July 2022)

Document History

First Codification	History	Date
Version#1	**KRIBIOLISA™ HEK HCP ELISA (Catalog No. KBBP15)**	20th September 2023

Approved Quality Control	Approved Product Development
Ankita G	Atul G

Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA™ HEK HCP ELISA (Catalog No. KBBP15).** 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**
- **Precision**
- **Recovery**
- **Linearity**
- **Stability**
- **Typical Data & Standard Curve**
- **Recovery by Spiking**
- **High Dose Hook Effect**
- **Dilutional Linearity**
- **Parallelism**

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 sales@krishgen.com.

1. Sensitivity

It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2* SD. 10 replicates of '0' standards were evaluated, and the sensitivity was found to be 46.875ng/ml.

2. Specificity / Cross reactivity:

Specificity of an analytical method is defined as its ability to measure an analyte accurately in the presence of interference. Antibodies used in the kit have been tested for reactivity with more than 50 proteins by Western Blot. Western Blot analysis against several strains of E.coli (XLI- Blue, DH5α, BL21, JM109, JM110, top 10, K12 & MC 1061) indicate that most of the proteins are conserved among all strains. Cross-reactivity has not been specifically investigated with this kit.

3. Precision

Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=20 assays) and inter-assay (n= 20 assays) reproducibility on two pools with low and high concentrations.

	Intra-assay Precision			**Inter-assay Precision**		
Sample Concentration	Low	Medium	High	Low	Medium	High

CV (%)	4.99	4.77	4.69	4.67	4.39	4.59

4. Recovery

A known amount of HEK 293 HCP were spiked into the sample matrix. Recovery was calculated by comparing the measured value with the expected amount of HEK 293 HCPs in the sample.

Matrix	Recovery Range (%)	Average (%)
Spiked Sample (n=10)	86-112	96

5. Linearity

The samples were diluted with a certain amount of HEK 293 HCPs at 1:2, 1:4 and 1:8 to understand linearity of dilution.

Sample	1:2	1:4	1:8
Diluted Samples (n=10)	95-105%	95-108%	91-101%

6. Stability:

An accelerated stability study set the shelf-life stability of **KRIBIOLISA™ HEK HCP ELISA (Catalog No. KBBP15)** was performed at 37°C and 2-8°C for 30 days and the full standard range was run on certain days. The kit was assessed to be stable for a period of 12 months with recoveries between the accepted 85% – 110%.

7. Parallelism:

Parallelism was assessed with multiple dilutions of samples that represent the same matrix and analyte combination. We have assayed the standard line along with the sample in linear dilution. The data given below shows both the standard line and the sample line to be parallel to each other with the slope being similar indicating sample-matrix has no substantial influence on sample estimates.

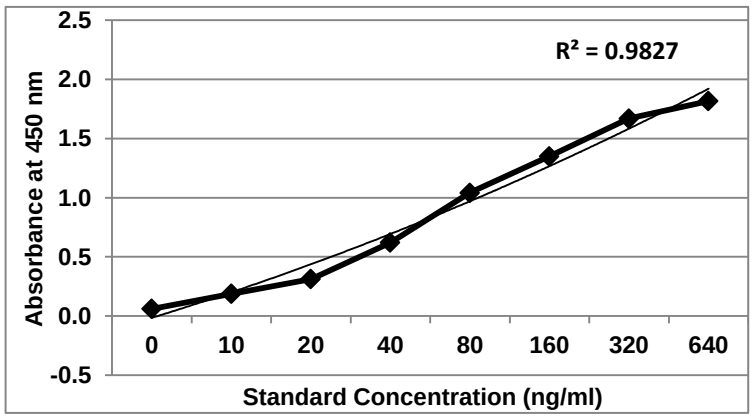
Standards (ng/ml)	Abs 1	Abs 2	Mean Absorbance	Corrected
0	0.069	0.073	0.071	0
78.13	0.109	0.114	0.111	0.04
156.25	0.153	0.161	0.156	0.085
312.5	0.182	0.191	0.185	0.115
625	0.374	0.393	0.382	0.311
1250	0.635	0.668	0.648	0.578
2500	1.288	1.354	1.315	1.244
5000	1.992	2.094	2.033	1.962

8. Typical Data

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual.

Standards (ng/ml)	Abs 1	Abs 2	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.069	0.073	0.071	0	0
78.13	0.109	0.114	0.111	85.2	109.0
156.25	0.153	0.161	0.156	218.5	139.8
312.5	0.182	0.191	0.185	289.2	92.5
625	0.374	0.393	0.382	688.2	110.1
1250	0.635	0.668	0.648	1174.6	94.0
2500	1.288	1.354	1.315	2550.7	102.0
5000	1.992	2.094	2.033	4977.2	99.5

Typical Graph



Note- * Abs = Absorbance at 450 nm. The controls are run in in-house buffers.
Absorbance Results are read on Tecan® Safire multimode reader and interpreted using GraphPad Prism® ver8.0

9. Lot-to-Lot Consistency

All lots are tested to ensure low background, a linear standard curve, consistent assay sensitivity, and a broad dynamic standard curve range. Consistent standard curve O.D.s and control values ensure that sample data is comparable over time.

This Lot is Passed.