VALIDATION OF KRIBIOLISA™ Panitumumab (VECTIBIX™) ELISA (Catalog No: KBI1082) 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 2018).

Document History

First Codification	History	Date
Version#1	**KRIBIOLISA™ Panitumumab (VECTIBIX™) ELISA (Catalog No: KBI1082)**	30th June 2023

Approved: Quality Control	Approved: Product Development
	
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Introduction

This document presents a discussion of the characteristics of our KRIBIOLISA™ Panitumumab (VECTIBIX™) ELISA (Catalog No: KBI1082). 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 - detection of Panitumumab**.**

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

- **Sensitivity**
- **Specificity**
- **Precision**
- **Cross Reactivity**
- **Stability**
- **Serum/Plasma Spike Recovery**
- **Hook Capacity**
- **Validation kit lot specific details**
- **Lot-to-Lot Consistency**

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

Panitumumab is a recombinant humanized monoclonal antibody used to treat EGFR-expressing, metastatic colorectal carcinoma. Panitumumab (ABX-EGF) is a recombinant human IgG2 monoclonal antibody that binds specifically to the human epidermal growth factor receptor (EGFR). Panitumumab was granted FDA approval on 27 September 2006.

Validation Information

1. Sensitivity:

Limit of Quantification**:** It is defined as the lowest concentration of an analyte that can be determined with an acceptable repeatability and the LOQ was found to be 8.30 ng/ml.

Limit Of Detection: 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 LOD was 7.75 ng/ml.

2. Specificity:

The antibodies used in the kit are monoclonal antibodies, anti-idiotypic and specific for Panitumumab. The calibrators / standards used are calibrated against commercially sourced (VECTIBIX™).

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=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (10 ng/ml), medium (80 ng/ml) and high (640 ng/ml) concentrations. While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Inter Assay %CV
Low	<10%	<10%
Medium	<5%	<5%
High	<5%	<5%

4. Cross-Reactivity:

No cross reactivity was observed.

5. Stability:

Shelf-Life Stability: An accelerated stability study set the shelf-life stability of KRIBIOLISA™ Panitumumab (VECTIBIX™) ELISA was performed, and a shelf life of 12 months was assigned to the kit.

6. Serum/Plasma Spike Recovery:

Normal human serum was used to spike at 640 ng/ml (being the highest standard) to check the recovery at different dilutions to observe optimal recoveries. It was seen that 1:100 serum dilution reported the most optimal recoveries.

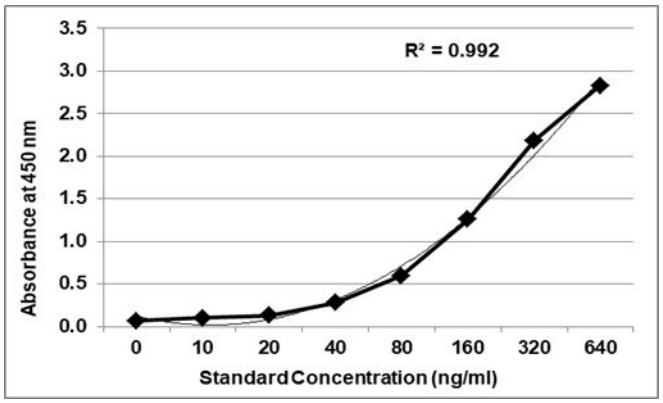
7. Hook Capacity:

The hook capacity, defined as that concentration that gives an absorbance reading less than the 640 ng/ml standard was ~640 ng/ml.

8. Validation Kit Lot Specific Details:

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.073	0.065	0.069	---	---
10	0.099	0.109	0.104	11.9	118.8
20	0.146	0.111	0.128	17.7	88.4
40	0.298	0.261	0.279	42.3	105.8
80	0.666	0.523	0.595	80.4	100.5
160	1.397	1.125	1.261	158.0	98.7
320	2.365	2.009	2.187	323.6	101.1
640	2.818	2.844	2.831	635.3	99.3

Typical Graph:



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.

The Lot is Passed.

Validation Guide Ver1.0 dated 01.7.2023