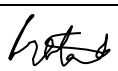
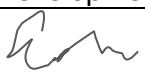
VALIDATION OF KRIBIOLISA™ Guselkumab (TREMFYA) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

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

Document History

First Codification	History	Date
Version#1	**KRIBIOLISA™ Guselkumab (TREMFYA) ELISA (Catalog NO. KBI1370)**	15th July 2023

Approved Quality Control	Approved Product Development
	
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Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA™ Guselkumab (TREMFYA) ELISA.** This kit has been validated as per EMA/FDA guidelines in line with ICH M10 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 Guselkumab.

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

- **Sensitivity**
- **Specificity**
- **Precision**
- **Traceability / Stability**
- **Validation kit 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 sales@krishgen.com

Background

Guselkumab is a human immunoglobulin G1 lambda (IgG1λ) monoclonal antibody that selectively blocks interleukin-23. IL-23 is an inflammatory cytokine that activates the CD4+ T-helper (Th17) cell pathway to mediate the inflammatory cascade that induces psoriatic plaque formation. In clinical trials, guselkumab demonstrated improved skin clearance and symptomatic improvements in dermatological manifestations of psoriasis. Developed by Janssen, the subcutenous injection form of guselkumab was approved in July 2017 under the market name Tremfya for the treatment of adult patients with moderate-to-severe plaque psoriasis.

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 9 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 8 ng/ml.

2. **Specificity**:

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

3. Precision:

3.1 Inter/Intra Assay 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 (160 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	<12%	<10%
Medium	<10%	<10%
High	<10%	<10%

4. Traceability and Stability:

4.1 Traceability:

The standards used in this kit have been calibrated against commercially sourced recombinant Guselkumab.

4.2 Stability:

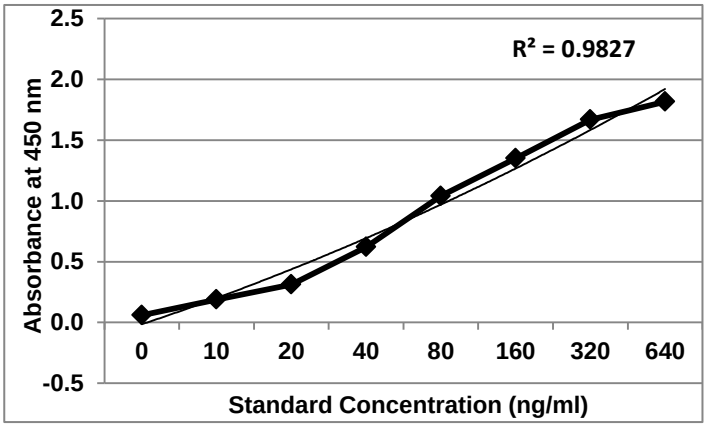
Shelf-Life Stability: An accelerated stability study set the shelf-life stability of **KRIBIOLISA™ Guselkumab (TREMFYA) ELISA** was performed, and a shelf life of 12 months was assigned to the kit.

5. Validation Kit Specific Details:

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.060	0.930	--
10	0.189	10.3	103.1
20	0.313	18.3	91.5
40	0.621	40.3	100.7
80	1.041	85.0	106.3
160	1.350	147.9	92.4
320	1.669	329.0	102.8
640	1.816	665.3	103.9

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

The Lot is Passed.

6. 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.

Validation Guide Ver1.0 dated 15.7.2023