ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF KRIBIOLISA® ANTI USTEKINUMAB (STELARA™) ELISA KIT (CATALOG NO. KBI2014) AS PER FDA/ICH GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA/ICH.

Document History

First Codification	History	Date
Version#1	**VALIDATION DATA OF KRIBIOLISA® ANTI USTEKINUMAB (STELARA™) ELISA (Cat No # KBI2014)**	31.07.2023

Approved Quality Control	Approved Product Development	Approved Operations Head
		
Prairna B	Atul G	K Jain

Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA® Anti-Ustekinumab (STELARA™) ELISA (Catalog No KBI2014)** kit 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 **Anti Ustekinumab.**

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

- **Assay Validation**
- **Standard Curve**
- **Pharmacokinetic Relevance**
- **Precision and Reproducibility**

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

Ustekinumab is a fully human monoclonal IgG1κ antibody that selectively targets the p40 subunit shared by the pro-inflammatory cytokines interleukin-12 (IL-12) and interleukin-23 (IL-23). By binding to this subunit, ustekinumab inhibits IL-12- and IL-23-mediated signaling, thereby reducing the activation of Th1 and Th17 pathways and suppressing inflammatory responses. Ustekinumab is developed for the treatment of moderate to severe plaque psoriasis, active psoriatic arthritis, and moderate to severe Crohn's disease in adult patients who have not responded adequately to conventional therapies. The drug is administered via subcutaneous injection and was approved by the U.S. Food and Drug Administration on September 25, 2009, under the brand name Stelara, providing a targeted immunomodulatory therapy for these chronic inflammatory conditions.

1. Purpose

To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Anti Ustekinumab (STELARA™) ELISA developed using Recombinant Ustekinumab as capture protein.

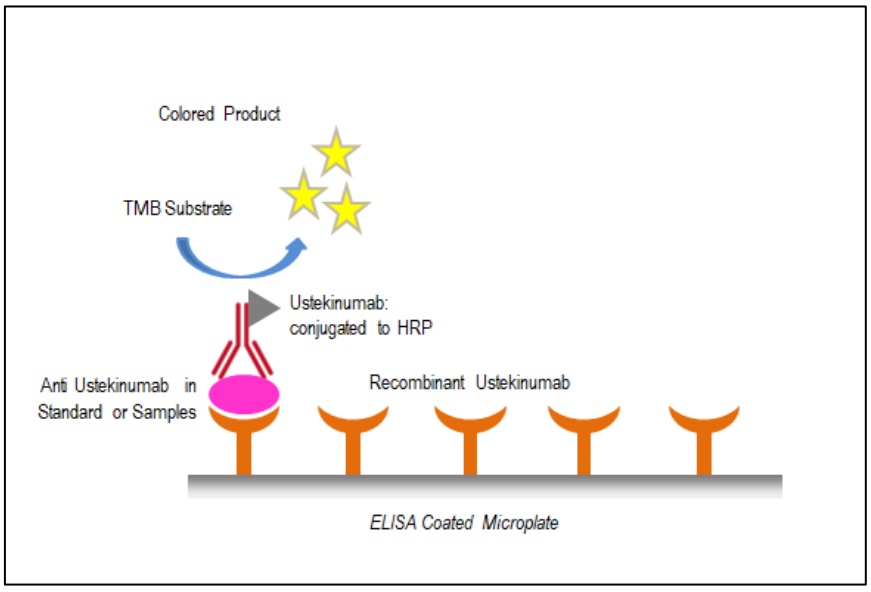
2. Experimental Design

- `A sandwich ELISA was performed using Recombinant Ustekinumab as capture protein.

- Standards prepared for Amivantamab.
- Assay Concentration Range: 0 - 640 ng/ml.
- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Ustekinumab ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant ustekinumab on the assay plate, thereby enhancing the selective binding of anti-ustekinumab antibodies. The immobilization procedure is designed to preserve the native conformation and epitope accessibility of ustekinumab, maintaining its structural integrity and functional orientation. This controlled presentation ensures that the antigen is displayed in a configuration that supports high-affinity interaction with the anti-ustekinumab binding domains.

Anti-ustekinumab antibodies demonstrate strong and stable antigen–antibody complex formation under these plate-bound conditions. In contrast, antibodies targeting unrelated proteins or different regions of ustekinumab may exhibit reduced or limited binding. This differential binding behavior reflects the high specificity of the assay and the preserved structural orientation of the immobilized ustekinumab established during the coating process.



ELISA kits for Anti Ustekinumab estimation offered by KRISHGEN uses Recombinant Ustekinumab capture proteins as above

3. Assay Validation

- IC50 Value: ~ 354.6 ng/ml (within 0-640 ng/mL assay range).
- LLOQ: ~ 7.86 ng/ml.

- Clinical Cmax Values*:
  - After a single 90 mg subcutaneous dose: ~5–7 µg/mL
  - After a single 45 mg subcutaneous dose: ~2–3 µg/mL
  - At steady state (following repeated dosing every 8–12 weeks, depending on indication): ~7–10 µg/mL

*Values are approximate and may vary depending on body weight, dosing schedule, and patient variability.

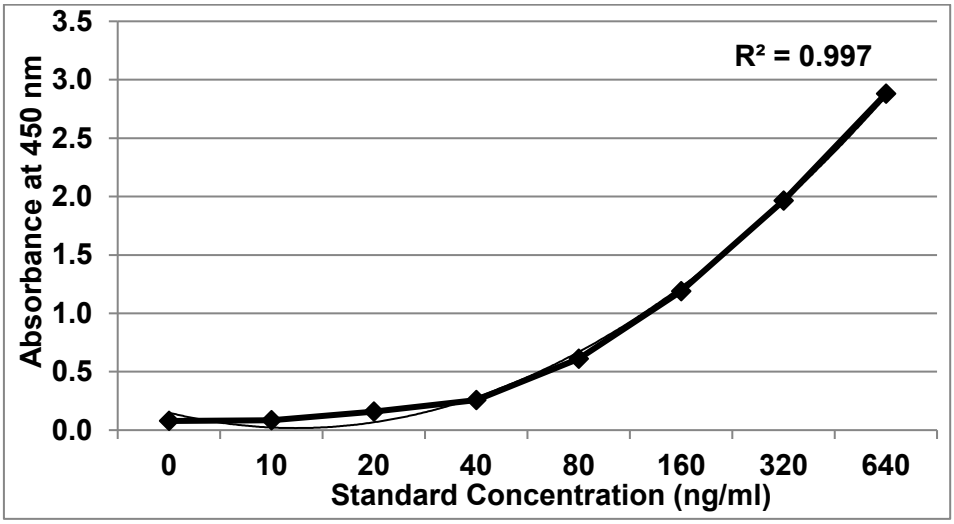
** published data*

- Precision:
  - Intra-Assay CV: <2%.
  - Inter-Assay CV: <4%.
  - Inter-Operator CV: <10%.

4. Standard Curve

Below is the standard curve for Anti Ustekinumab Sandwich ELISA assay:
Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.079	--	--
10	0.093	8.1	81.0
20	0.160	20.2	100.9
40	0.258	34.6	86.4
80	0.611	81.8	102.3
160	1.190	164.6	102.9
320	1.965	313.4	97.9
640	2.879	644.6	100.7



5. LOD and LOQ

- LOD Absorbance: (Approx ~2.59 ng/ml)
- LOQ Absorbance: (Approx ~7.86 ng/ml)

6. Pharmacokinetic Relevance

The assay is designed to cover the clinically relevant serum concentrations of anti-ustekinumab antibodies observed following subcutaneous therapeutic dosing, making it suitable for pharmacokinetic evaluation and therapeutic monitoring. The anti-ustekinumab ELISA demonstrates sensitivity within the µg/mL range, which falls well within the validated assay limits, ensuring accurate quantification across clinically meaningful exposure levels.

Published pharmacokinetic data indicate systemic exposure consistent with therapeutic monoclonal antibodies:
• Following recommended subcutaneous dosing regimens (e.g., 45–90 mg every 8–12 weeks, depending on indication), the reported initial Cmax ranges from approximately 2–7 µg/mL.
• With repeated dosing, steady-state peak concentrations typically reach approximately 7–10 µg/mL.
• Actual exposure may vary depending on body weight, dosing frequency, and individual patient variability.
Thus:
• At clinically relevant subcutaneous doses, anti-ustekinumab serum concentrations fall within the measurable range of the ELISA following appropriate sample dilution.
• The assay working range enables reliable differentiation across varying systemic exposure levels.
• Routine dilution of clinical samples is recommended to ensure measurements fall within the linear dynamic range of the assay.
• The assay is therefore suitable for pharmacokinetic profiling, dose–exposure analysis, and therapeutic monitoring of anti-ustekinumab in human serum or plasma.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (20 ng/ml, 80 ng/ml, and 640 ng/ml). Each concentration was tested in triplicate across three independent assay runs. %CV (Coefficient of Variation) was calculated within runs (intra-assay precision) and across runs (inter-assay precision).

Acceptance Criteria:

- Intra-assay %CV should be ≤15% for samples.
- Inter-assay %CV should be ≤15% for samples.
- %CV at LLOQ (Lower Limit of Quantitation) allowed up to 20%.

Precision Results Summary:

Standard (ng/ml)	Intra-Assay %CV (Range)	Inter-Assay %CV
20	2.2 % to 4.5%	<2%
80	1% to 5.4%	<4%
640	0.3% to 2%	<1%

Observations:

- Intra-assay precision was consistently less than 6% across all levels tested.
- Inter-assay precision was consistently less than 4%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:
The KRIBIOLISA® Anti Ustekinumab (STELARA™) ELISA demonstrates excellent intra- and inter-assay precision. These results support the assay's reliability and reproducibility for routine use in pharmacokinetic and bioanalytical studies.

8. Conclusion

The KRIBIOLISA® Anti-Ustekinumab (STELARA™) ELISA is validated for sensitivity, specificity, precision, and accuracy, and is appropriate for pharmacokinetic applications in regulatory settings.

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Version 1.0, dated 31.07.2023