ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF KRIBIOLISA® CERTOLIZUMAB PEGOL (CIMZIA™) INHIBITION ELISA KIT (CATALOG NO. KBI1060) 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® CERTOLIZUMAB PEGOL (CIMZIA™) INHIBITION ELISA (Cat No # KBI1060)**	31.01.2026

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® Certolizumab pegol (CIMZIA™) Inhibition ELISA (Catalog No KBI1060)** 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 **Certolizumab pegol.**

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

Certolizumab pegol is a pegylated, humanised antigen-binding fragment (Fab') of a monoclonal antibody that specifically targets tumour necrosis factor-alpha (TNF-α), a key pro-inflammatory cytokine involved in the pathogenesis of several chronic inflammatory and autoimmune diseases. By binding with high affinity to both soluble and membrane-bound TNF-α, certolizumab pegol neutralizes its biological activity and inhibits downstream inflammatory signaling, thereby reducing inflammation and tissue damage. Unlike full-length antibodies, certolizumab pegol lacks an Fc region and is conjugated to polyethylene glycol (PEG), which prolongs its circulation time and reduces immune-mediated effector functions. It is indicated for the treatment of conditions such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis, and Crohn's disease, and is administered by subcutaneous injection as a targeted anti-TNF therapy for long-term management of immune-mediated inflammatory disorders.

1. Purpose

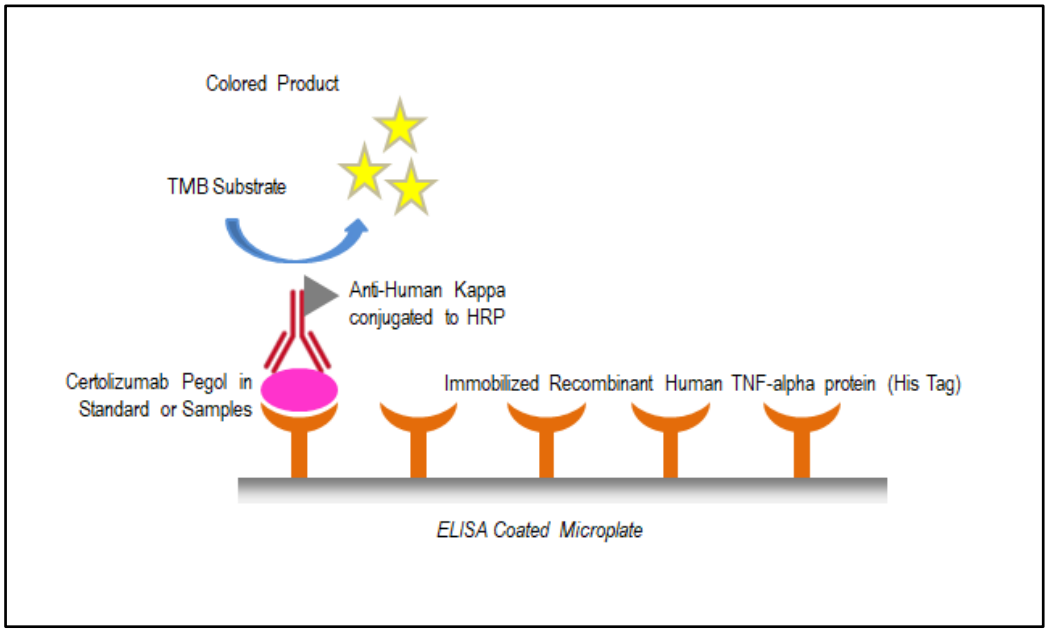
To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Certolizumab pegol (CIMZIA™) Inhibition ELISA developed using Recombinant Human TNF-alpha protein (His Tag) as capture protein.

2. Experimental Design

- `A indirect ELISA was performed using Recombinant Human TNF-alpha protein (His Tag) as capture protein.
- Standards prepared for Certolizumab pegol.
- Assay Concentration Range: 0 - 1000 ng/ml.
- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Certolizumab Pegol ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant human tumour necrosis factor-alpha (TNF-α) on the assay plate, thereby enhancing the selective binding of Certolizumab Pegol. The immobilization process is designed to preserve the native conformation and epitope accessibility of TNF-α, maintaining its structural integrity and biological activity. This approach ensures that the antigen is presented in a configuration that supports high-affinity interaction with the TNF-α-specific binding sites of Certolizumab Pegol.

Certolizumab Pegol binds with high specificity and affinity to both soluble and immobilized TNF-α, resulting in stable antigen–antibody complex formation. Other monoclonal antibodies directed against unrelated cytokines, inflammatory mediators, or proteins outside the TNF pathway may exhibit reduced or minimal binding under these plate-bound conditions. This differential binding behavior reflects the TNF-α specificity of Certolizumab Pegol as well as the controlled conformation and orientation of the immobilized TNF-α antigen established during the immobilization process.



ELISA kits for Certolizumab Pegol estimation offered by KRISHGEN uses Recombinant Human TNF-alpha protein (His Tag) capture proteins as above

3. Assay Validation

- IC50 Value: ~ 223.9 ng/ml (within 0-1000 ng/mL assay range).
- LLOQ: ~ 14.3 ng/ml.

- Clinical Cmax Values*:
  - Following subcutaneous administration in clinical studies (typical dosing range 200–400

mg), peak serum concentrations were achieved within several days, with Cmax values generally ranging from approximately 20 to 50 µg/mL, depending on the dose and dosing schedule.

- After repeated subcutaneous dosing (e.g., 400 mg at Weeks 0, 2, and 4 followed by 200 mg every 2 weeks or 400 mg every 4 weeks), observed peak concentrations were generally in the range of ~30–60 µg/mL.
- At steady state following multiple-dose administration, Cmax values typically ranged between ~40–70 µg/mL, reflecting accumulation consistent with the pharmacokinetic profile and extended half-life of the PEGylated antibody fragment.

*Values are approximate and may vary depending on dose level, dosing schedule, patient population, tumour burden, and inter-individual pharmacokinetic variability.

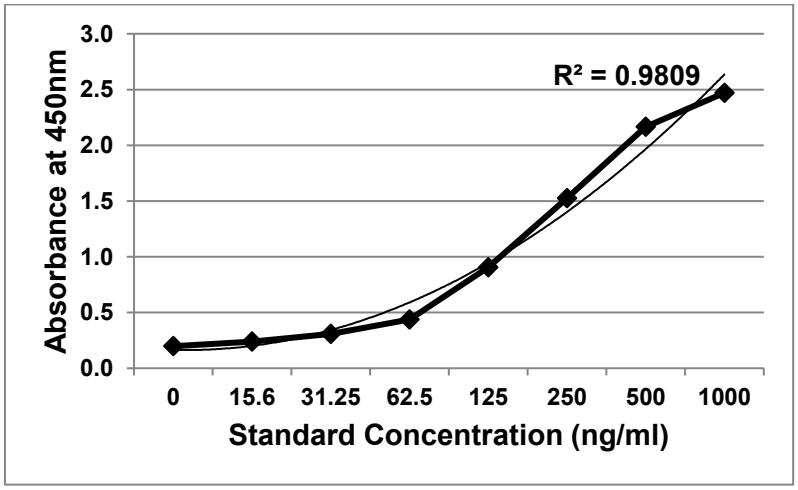
* *published data*

- Precision:
  - Intra-Assay CV: <8%.
  - Inter-Assay CV: <5%.
  - Inter-Operator CV: <10%.

4. Standard Curve

Below is the standard curve for Certolizumab Pegol Indirect ELISA assay:
Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	%Interpolated Concentration against Actual Concentration
0	0.197	--	--
15.6	0.239	17.5	112.2
31.25	0.309	34.5	110.5
62.5	0.437	57.7	92.3
125	0.911	129.1	103.3
250	1.524	244.9	98.0
500	2.167	511.1	102.2
1000	2.470	981.4	98.1



5. LOD and LOQ

- LOD Absorbance: (Approx ~6.73 ng/ml)
- LOQ Absorbance: (Approx ~14.39 ng/ml)

6. Pharmacokinetic Relevance

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

Published pharmacokinetic data for Certolizumab pegol indicate systemic exposure consistent with PEGylated anti-TNF biologics administered subcutaneously: Following the recommended induction regimen (400 mg at Weeks 0, 2, and 4), peak serum concentrations (Cmax) are generally observed in the range of approximately 30–50 µg/mL.

• With continued maintenance dosing (200 mg every 2 weeks or 400 mg every 4 weeks), steady-state peak concentrations typically reach approximately 40–70 µg/mL.

• Exposure levels may vary depending on indication, body weight, concomitant immunosuppressive therapy, dosing schedule, and inter-individual pharmacokinetic variability.

Thus:

• At clinically relevant subcutaneous doses, Certolizumab pegol serum concentrations fall within the measurable range of the ELISA following appropriate sample dilution.

• The assay working range enables reliable quantification across varying systemic exposure levels.

• Routine dilution of clinical samples is recommended, where necessary, 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 Certolizumab pegol in human serum or plasma.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (15.6 ng/ml, 125 ng/ml, and 1000 ng/m). 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 (ug/ml)	Intra-Assay %CV (Range)	Inter-Assay %CV
15.6	3.8% to 6.2%	<6%
125.0	1% to 6%	<6%
1000	0.8% to 4.3%	<4%

Observations:

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

Conclusion:
The KRIBIOLISA® Certolizumab pegol (CIMZIA™) Inhibition 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® Certolizumab pegol (CIMZIA™) Inhibition ELISA is validated for sensitivity, specificity, precision, and accuracy, and is appropriate for pharmacokinetic applications in regulatory settings.

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