ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF KRIBIOLISA® AMIVANTAMB (RYBREVANT™) ELISA KIT (CATALOG NO. KBI1130) 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® AMIVANTAMB (RYBREVANT™) ELISA (Cat No # KBI1130)**	30.09.2025

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® Amivantamab (RYBREVANT™) ELISA (Catalog No KBI1130)** 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 **Amivantamab.**

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

Amivantamab is a fully human bispecific IgG1 monoclonal antibody targeting both epidermal growth factor receptor (EGFR) and mesenchymal–epithelial transition factor (MET). By binding to the extracellular domains of these receptors, it inhibits ligand-dependent signaling, promotes receptor degradation, and induces immune-mediated tumor cell killing. Amivantamab is developed for the treatment of non-small cell lung cancer (NSCLC), specifically in adult patients with locally advanced or metastatic disease harboring EGFR exon 20 insertion mutations that have progressed on or after platinum-based chemotherapy. The drug is administered via intravenous infusion and was approved by the US Food and Drug Administration (FDA) on May 21, 2021, under the brand name RYBREVANT™, providing the first targeted therapy option for this difficult-to-treat genetic subtype of NSCLC.

1. Purpose

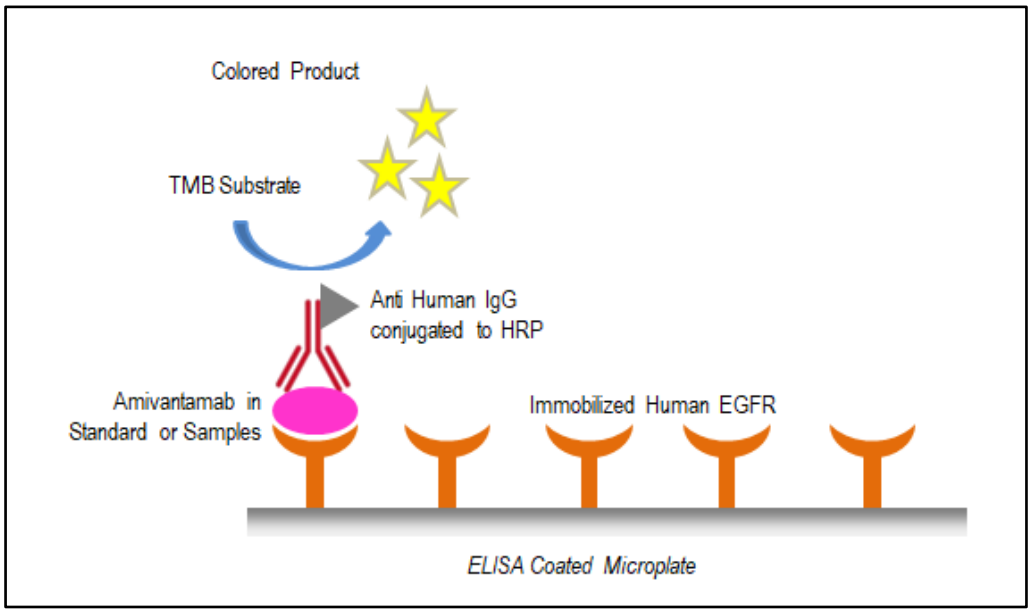
To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Amivantamab (RYBREVANT™) ELISA developed using Human EGFR as capture protein.

2. Experimental Design

- `A sandwich ELISA was performed using Human EGFR as capture protein.

- Standards prepared for Amivantamab.
- Assay Concentration Range: 0 - 500 ng/ml.
- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Amivantamab ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant extracellular domains of EGFR and/or MET on the assay plate, thereby enhancing the selective binding of Amivantamab. The immobilization procedure is designed to preserve the native conformation and epitope accessibility of the receptor proteins, maintaining their structural integrity and functional orientation. This approach ensures that the antigen is presented in a configuration that supports high-affinity interaction with amivantamab's dual-binding domains.
Amivantamab's bispecific structure enables simultaneous and high-affinity recognition of EGFR and MET epitopes, resulting in strong and stable antigen–antibody complex formation. In contrast, other monoclonal antibodies that target only EGFR or MET individually, or that recognize different epitopes within these receptors, may demonstrate reduced or limited binding under these plate-bound conditions. This differential binding behaviour reflects the unique dual-target specificity of Amivantamab as well as the controlled conformation and orientation of the immobilized receptor antigens established during the immobilization process.



ELISA kits for Amivantamab estimation offered by KRISHGEN uses Human EGFR capture proteins as above

3. Assay Validation

- IC50 Value: ~ 99.2 ng/ml (within 0-500 ng/mL assay range).
- LLOQ: ~ 3.07 ng/ml.

- Clinical Cmax Values*:
  - After 350 mg dose (<80 kg body weight): ~80–90 µg/mL
  - After 700 mg dose (≥80 kg body weight): ~160–180 µg/mL
  - At steady state (after repeated weekly then biweekly dosing): ~250–320 µg/mL

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

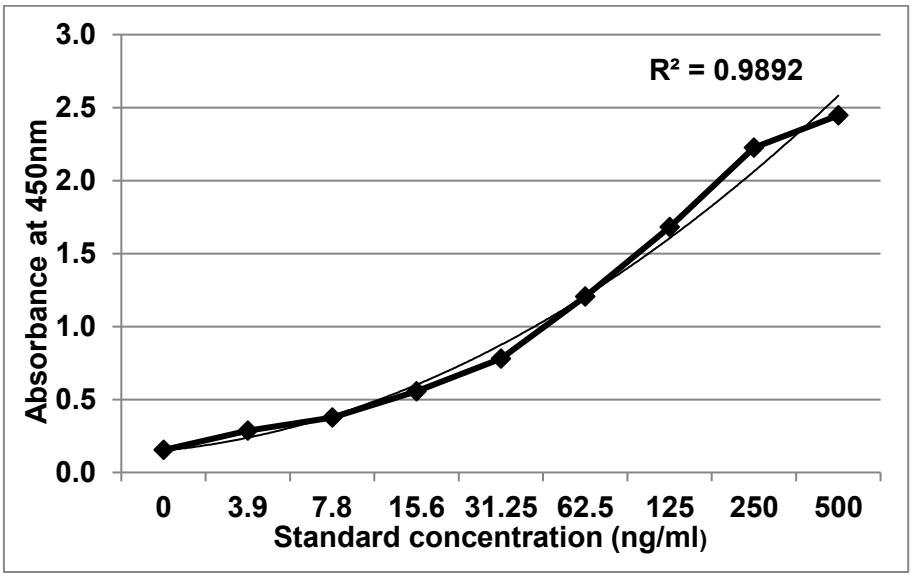
** published data*

- Precision:
  - Intra-Assay CV: <7%.
  - Inter-Assay CV: <8%.
  - Inter-Operator CV: <10%.

4. Standard Curve

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

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	%Interpolated Concentration against Actual Concentration
0	0.154	--	---
3.9	0.287	4.6	116.9
7.8	0.376	8.5	109.5
15.6	0.556	17.2	110.0
31.25	0.780	29.6	94.9
62.5	1.206	61.2	97.9
125	1.682	121.6	97.2
250	2.225	279.8	111.9
500	2.447	458.6	91.7



5. LOD and LOQ

- LOD Absorbance: (Approx ~1.01 ng/ml)
- LOQ Absorbance: (Approx ~3.07 ng/ml)

6. Pharmacokinetic Relevance

The assay is designed to cover the clinically relevant serum concentrations of Amivantamab observed following intravenous therapeutic dosing, making it suitable for pharmacokinetic evaluation and therapeutic monitoring.

The Amivantamab 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 Amivantamab indicate systemic exposure consistent with therapeutic monoclonal antibodies:

• Following the recommended intravenous dosing regimen (weight-based: 350 mg for patients <80 kg and 700 mg for patients ≥80 kg), the reported initial Cmax is approximately 80–180 µg/mL.

• With repeated dosing (weekly for the initial cycles followed by every two weeks), steady-state peak concentrations typically reach approximately 250–320 µg/mL.

• Higher exposure levels may be observed depending on body weight, dosing frequency, and individual patient variability.

Thus:

• At clinically relevant intravenous doses, Amivantamab 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.

• Given the relatively high circulating concentrations associated with therapeutic dosing, 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 Amivantamab in human serum or plasma.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (3.9 ng/ml, 62.5 ng/ml, and 500 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
3.9	1.8% to 8%	<4%
62.5	0.9% to 3.7%	<4%
500	0.7% to 1.9%	<2%

Observations:

- Intra-assay precision was consistently less than 4% 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® Amivantamab (RYBREVANT™) 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® Amivantamab (RYBREVANT™) ELISA is validated for sensitivity, specificity, precision, and accuracy, and is appropriate for pharmacokinetic applications in regulatory settings.

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