# ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

**VALIDATION OF KRIBIOLISA® BIMAGRUMAB ELISA KIT (CATALOG NO. KBI1192) 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® BIMAGRUMAB ELISA (Cat No # KBI1192)** | 31.12.2025 |
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| Approved Quality Control | Approved Product Development | Approved Operations Head |
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| Prairna B | Atul G | K Jain |

## Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA® Bimagrumab ELISA (Catalog No KBI1192)** 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 **Bimagrumab.**

**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

Bimagrumab is a fully human IgG1 monoclonal antibody that targets activin type II receptors (ActRIIA and ActRIIB), key regulators of skeletal muscle growth and body composition. These receptors mediate signaling of ligands such as myostatin and activins, which normally inhibit muscle development. By binding to ActRII receptors and blocking these inhibitory pathways, Bimagrumab promotes skeletal muscle hypertrophy, increases lean body mass, and reduces fat mass, leading to improvements in strength and metabolic function. The therapy has been investigated for conditions associated with muscle loss and adverse body composition, including sarcopenia, muscle wasting disorders, obesity, and type 2 diabetes. Bimagrumab is administered by intravenous infusion and represents a targeted biological approach aimed at improving muscle function and metabolic health, although its regulatory approval status varies depending on the indication and region.

## 1. Purpose

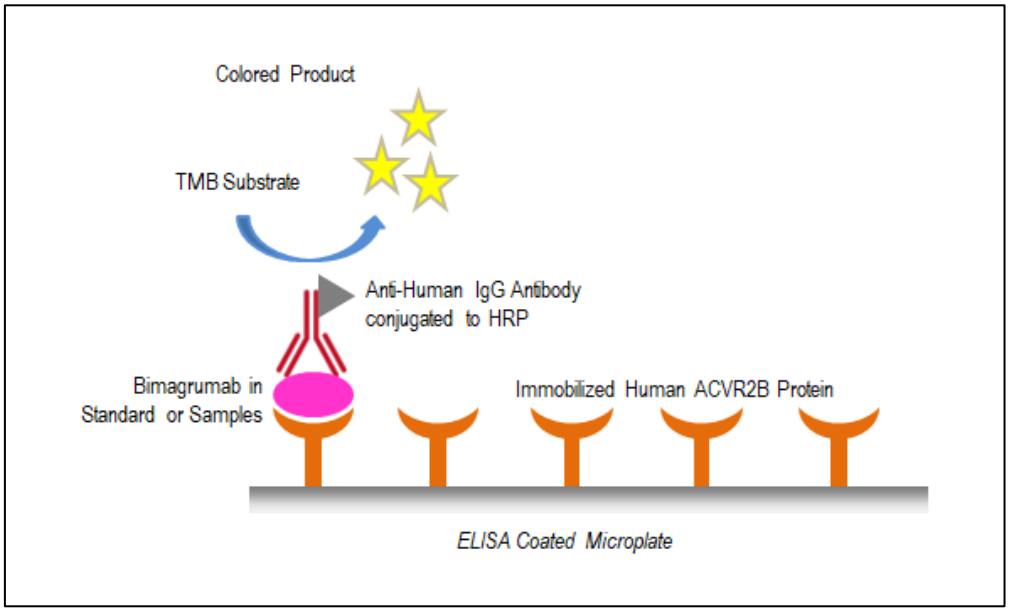
To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Bimagrumab ELISA developed using Recombinant Human ACVR2B Protein as capture protein.

## 2. Experimental Design

- `A sandwich ELISA was performed using Recombinant Human ACVR2B Protein as capture protein.
- Standards prepared for Bimagrumab.
- Assay Concentration Range: 0 - 1000 ng/ml.
- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Bimagrumab ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant human activin type II receptor (ActRIIA/ActRIIB) extracellular domain on the assay plate, thereby enhancing the selective binding of Bimagrumab. The immobilization process is designed to preserve the native conformation and epitope accessibility of the receptor, maintaining its structural integrity and functional orientation. This approach ensures that the antigen is presented in a configuration that supports high-affinity interaction with Bimagrumab's ActRII-specific binding sites.

Bimagrumab binds with high specificity and affinity to activin type II receptors, resulting in stable antigen–antibody complex formation. Other monoclonal antibodies directed against unrelated growth factors, cytokines, or components of the transforming growth factor-beta (TGF-β) superfamily that do not target ActRIIA/ActRIIB may exhibit reduced or minimal binding under these plate-bound conditions. This differential binding behaviour reflects the receptor specificity of Bimagrumab as well as the controlled conformation and orientation of the immobilized ActRII antigen established during the immobilization process.




ELISA kits for Bimagrumab estimation offered by KRISHGEN uses Recombinant Human ACVR2B capture proteins as above

## 3. Assay Validation

- IC50 Value: ~ 217.2 ng/ml (within 0-250 ng/mL assay range).
- LLOQ: ~ 16.30 ng/ml.

- Clinical Cmax Values*:
  - Following single intravenous doses evaluated in clinical studies (dose range 1–30 mg/kg):

peak serum concentrations increased in a dose-proportional manner, with typical Cmax values ranging from approximately 20 to 600 µg/mL, depending on the administered dose.
- After repeated intravenous dosing (e.g., 10 mg/kg every 4 weeks), observed peak concentrations were generally in the range of ~200–400 µg/mL.
- At steady state following multiple-dose administration, Cmax values typically ranged between ~300–500 µg/mL, reflecting accumulation consistent with the antibody's elimination half-life.

*Values are approximate and may vary depending on dose level, dosing frequency, patient population, and clinical indication.

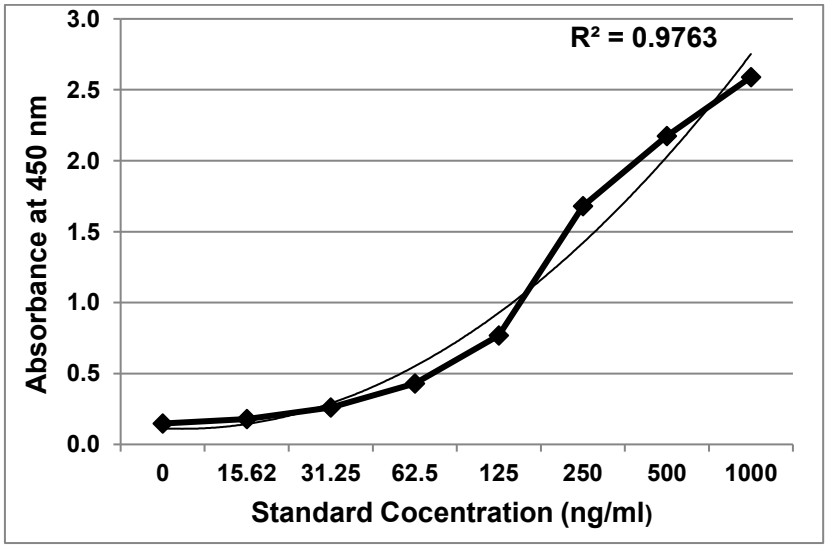
* *published data*

- Precision:
  - Intra-Assay CV: <4%.
  - Inter-Assay CV: <5%.
  - Inter-Operator CV: <10%.

## 4. Standard Curve

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

| Standard Concentration (ng/ml) | Mean Absorbance | Interpolated Concentration | %Interpolated Concentration against Actual Concentration |
|---|---|---|---|
| 0 | 0.147 | -- | -- |
| 15.62 | 0.178 | 12.8 | 82.0 |
| 31.25 | 0.262 | 37.3 | 119.3 |
| 62.5 | 0.429 | 67.1 | 107.4 |
| 125 | 0.768 | 114.9 | 91.9 |
| 250 | 1.680 | 269.3 | 107.7 |
| 500 | 2.173 | 450.3 | 90.1 |
| 1000 | 2.590 | 1159.4 | 115.9 |



## 5. LOD and LOQ

- LOD Absorbance: (Approx ~5.38 ng/ml)
- LOQ Absorbance: (Approx ~16.30 ng/ml)

## 6. Pharmacokinetic Relevance

The assay is designed to cover the clinically relevant serum concentrations of Bimagrumab observed following intravenous therapeutic dosing, making it suitable for pharmacokinetic evaluation and therapeutic monitoring. The Bimagrumab 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 Bimagrumab indicate systemic exposure consistent with intravenously administered therapeutic monoclonal antibodies:

• Following single intravenous doses (1–30 mg/kg), peak serum concentrations (Cmax) increase in a dose-proportional manner and may range from approximately 20 to 600 µg/mL, depending on the dose level.

• With repeated dosing regimens (e.g., 10 mg/kg every 4 weeks), steady-state peak concentrations are typically observed in the range of approximately 300–500 µg/mL.

• Exposure levels may vary based on dose, dosing interval, clinical indication, body composition, and inter-individual pharmacokinetic variability.

Thus:

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

• The assay working range enables reliable quantification across a broad range of 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 Bimagrumab in human serum or plasma.

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## 7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (15.6 ng/ml, 125 ng/ml, and 1000 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 |
|---|---|---|
| 15.6 | 1.9% to 4.5% | <5% |
| 125 | 2.5% to 9.3% | <9% |
| 1000 | 1.7% to 2.6% | <3% |

Observations:

- Intra-assay precision was consistently less than 5% across all levels tested.
- Inter-assay precision was consistently less than 9%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:
The KRIBIOLISA® Bimagrumab 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® Bimagrumab ELISA is validated for sensitivity, specificity, precision, and accuracy, and is appropriate for pharmacokinetic applications in regulatory settings.**

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