ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF KRIBIOLISA® BUROSUMAB (CRYSVITA™) ELISA KIT (CATALOG NO. KBI1209) 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® BUROSUMAB (CRYSVITA™) ELISA (Cat No # KBI1209)**	31.07.2025

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® Burosumab (CRYSVITA™) ELISA (Catalog No KBI1209)** 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 **Burosumab.**

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

Burosumab (Crysvita™) is a fully human IgG1 monoclonal antibody that targets fibroblast growth factor 23 (FGF23), a hormone involved in phosphate regulation. By binding to and inhibiting the activity of excess FGF23, burosumab increases renal tubular phosphate reabsorption and enhances the production of active vitamin D, thereby improving serum phosphate levels and promoting bone mineralisation. It is indicated for the treatment of X-linked hypophosphataemia (XLH) in paediatric and adult patients, as well as for tumour-induced osteomalacia (TIO) associated with phosphaturic mesenchymal tumours that cannot be curatively resected or localised, depending on regional regulatory approvals. Burosumab is administered by subcutaneous injection and represents a targeted therapy for the management of chronic hypophosphataemic disorders caused by excess FGF23 activity.

1. Purpose

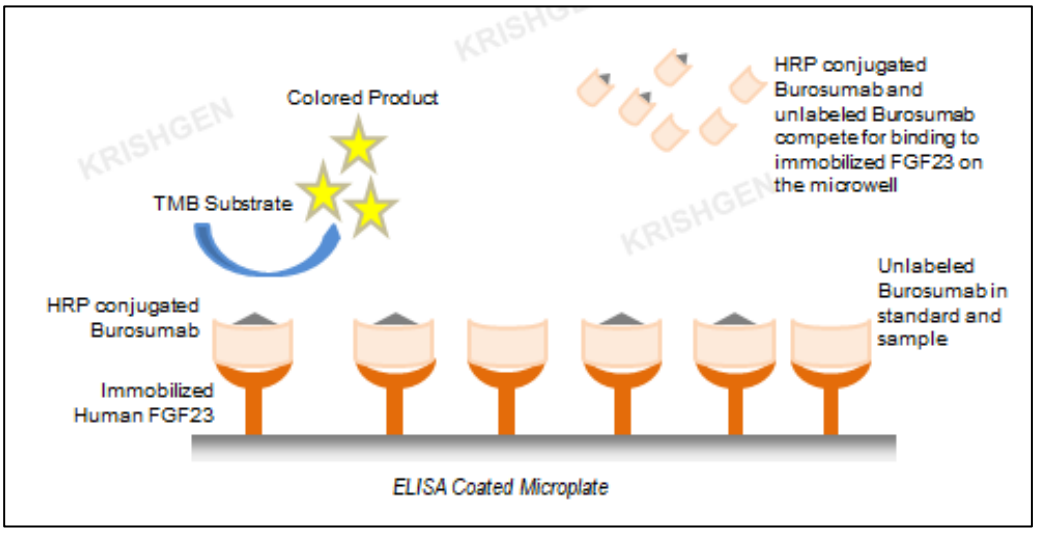
To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Burosumab (CRYSVITA™) ELISA developed using rHFGF-23 Human Protein as capture protein.

2. Experimental Design

- `A competitive ELISA was performed using rHFGF-23 Human Protein as capture protein.
- Standards prepared for Blinatumomab.
- Assay Concentration Range: 0 – 32 ug/ml.
- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Burosumab ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant human fibroblast growth factor 23 (FGF23) on the assay plate, thereby enhancing the selective binding of Burosumab. The immobilization process is designed to preserve the native conformation and epitope accessibility of FGF23, maintaining its structural integrity and functional orientation. This approach ensures that the antigen is presented in a configuration that supports high-affinity interaction with the FGF23-specific binding sites of Burosumab.

Burosumab binds with high specificity and affinity to FGF23, resulting in stable antigen–antibody complex formation under the assay conditions. Other monoclonal antibodies directed against different endocrine, metabolic, or unrelated targets, or antibodies not specific to FGF23, may exhibit reduced or minimal binding under these plate-bound conditions. This differential binding behaviour reflects the FGF23 specificity of Burosumab as well as the controlled conformation and orientation of the immobilized FGF23 antigen established during the immobilization process.



ELISA kits for Burosumab estimation offered by KRISHGEN uses rHFGF-23 Human capture proteins as above

3. Assay Validation

- IC50 Value: ~ 2.845 ug/ml (within 0- 32 ug/ml assay range).
- LLOQ: ~ 3.26 ug/ml.

- Clinical Cmax Values*:
 - Following the recommended dosing regimen (subcutaneous administration at 0.8–1 mg/kg every 2 weeks in paediatric patients or 1 mg/kg every 4 weeks in adults): ~5–15 µg/mL
 - After repeated dosing (steady-state conditions): ~10–20 µg/mL
 - Trough concentrations prior to the next dose at steady state: ~2–8 µg/mL

*Values are approximate and may vary depending on age, body weight, dosing interval, disease severity, renal function, treatment duration, and inter-patient variability.

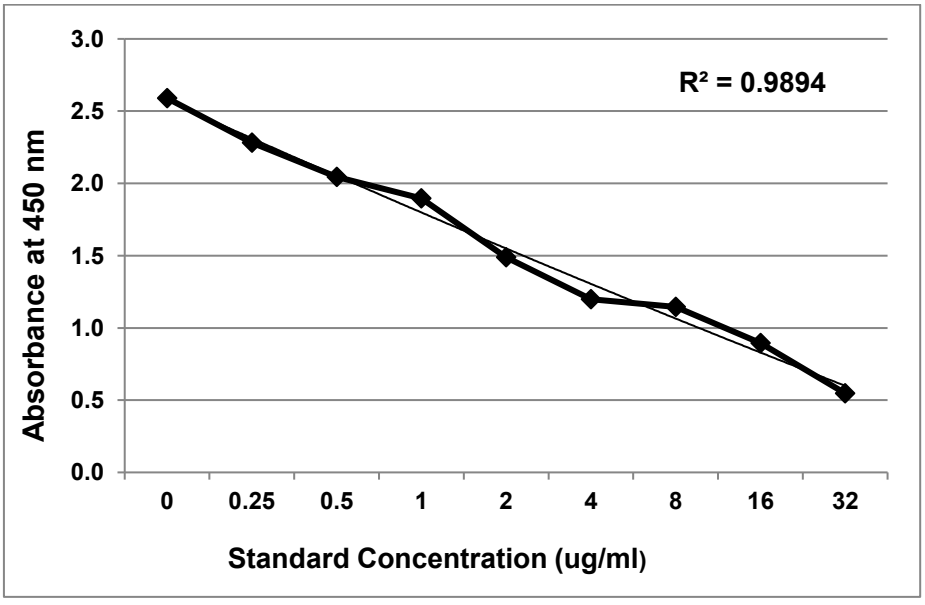
* *published data*

- Precision:
  - Intra-Assay CV: <5%.
  - Inter-Assay CV: <6%.
  - Inter-Operator CV: <10%.

4. Standard Curve

Below is the standard curve for Burosumab competitive ELISA assay:
Linearity and Range

Standard Concentration (ug/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	2.590	0.1	--
0.25	2.281	0.3	102.1
0.5	2.045	0.5	101.5
1	1.896	0.8	78.1
2	1.488	2.5	126.2
4	1.199	4.9	121.7
8	1.146	6.7	83.6
16	0.896	13.6	84.8
32	0.547	36.3	113.4



5. LOD and LOQ

- LOD Absorbance: (Approx ~1.08 ug/ml)
- LOQ Absorbance: (Approx ~3.26 ug/ml)

6. Pharmacokinetic Relevance

The assay is designed to cover the clinically relevant serum concentrations of Burosumab observed following subcutaneous therapeutic administration, making it suitable for pharmacokinetic evaluation and therapeutic monitoring. The Burosumab ELISA demonstrates sensitivity within the µg/mL range, which aligns with the circulating concentrations of this therapeutic monoclonal antibody and ensures accurate quantification across clinically meaningful exposure levels.

Published pharmacokinetic data for Burosumab indicate systemic exposure consistent with subcutaneously administered IgG monoclonal antibodies:

• Following the recommended dosing regimen (approximately 0.8–1 mg/kg every 2 weeks in paediatric patients or 1 mg/kg every 4 weeks in adults), peak serum concentrations (Cmax) are typically in the range of approximately 5–15 µg/mL.

• With repeated dosing, steady-state peak concentrations generally reach approximately 10–20 µg/mL.

• Exposure levels may vary depending on age, body weight, dosing interval, disease severity, renal function, treatment duration, and inter-patient variability.

Thus:

• At clinically relevant subcutaneous doses, Burosumab 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, 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 Burosumab in human serum or plasma.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (0.25 ug/ml, 2 ug/ml, and 32 ug/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 (ug/ml)	Intra-Assay %CV (Range)	Inter-Assay %CV
0.25	0.3% to 1.8%	<2%
2	0.5% to 3.8%	<4%
32	2.1% to 5.1%	<6%

Observations:

- Intra-assay precision was consistently less than 5% 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® Burosumab (CRYSVITA™) 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® Burosumab (CRYSVITA™) 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 31.07.2025