ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

VALIDATION OF KRIBIOLISA® BLINATUMOMAB (BLINCYTO™) ELISA KIT (CATALOG NO. KBI1197) 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® BLINATUMOMAB (BLINCYTO™) ELISA (Cat No # KBI1197)**	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® Blinatumomab (BLINCYTO™) ELISA (Catalog No KBI1197)** 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 **Blinatumomab.**

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

Blinatumomab is a bispecific T-cell engager (BiTE®) antibody construct that simultaneously targets CD19, a surface antigen expressed on B cells, and CD3, a component of the T-cell receptor complex. By binding to CD19-positive B cells and CD3-positive T cells, blinatumomab brings cytotoxic T lymphocytes into close proximity with malignant B cells, resulting in T-cell activation, proliferation, and targeted lysis of CD19-expressing tumour cells. It is indicated for the treatment of patients with relapsed or refractory B-cell precursor acute lymphoblastic leukaemia (ALL), including those with minimal residual disease (MRD)-positive disease, depending on regional regulatory approvals. Blinatumomab is administered as a continuous intravenous infusion due to its short serum half-life and represents an important immunotherapy option for the management of CD19-positive B-cell malignancies.

1. Purpose

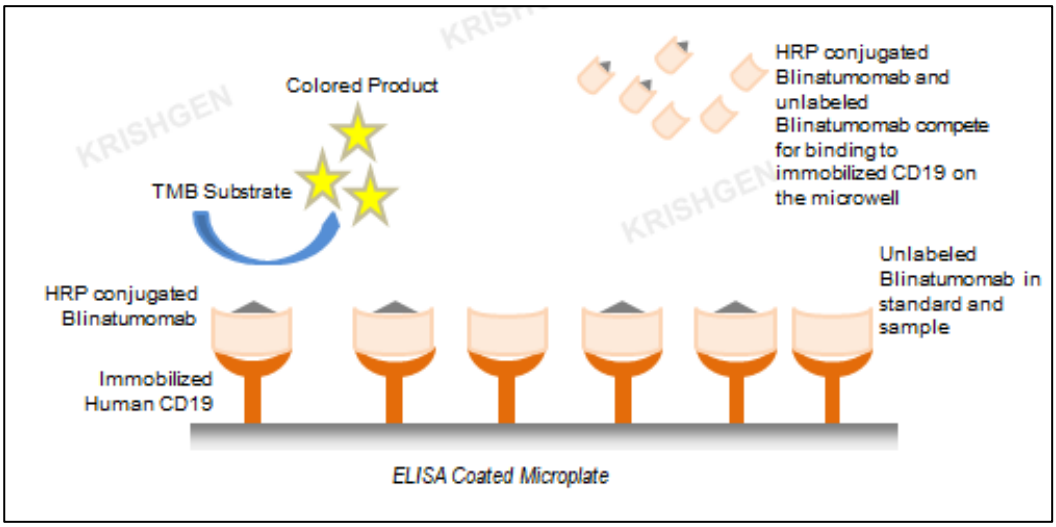
To assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Blinatumomab (BLINCYTO™) ELISA developed using Human CD19/Leu-12 Protein as capture protein.

2. Experimental Design

- `A competitive ELISA was performed using Human CD19/Leu-12 Protein as capture protein.
- Standards prepared for Blinatumomab.
- Assay Concentration Range: 0 - 6000 ng/ml.
- Signal (% absorbance) plotted versus concentration.

The KRIBIOLISA Blinatumomab ELISA employs a targeted immobilization strategy to ensure optimal presentation of recombinant human CD19 antigen on the assay plate, thereby enhancing the selective binding of Blinatumomab. The immobilization process is designed to preserve the native conformation and epitope accessibility of CD19, 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 CD19-specific binding domain of Blinatumomab.

Blinatumomab binds with high specificity and affinity to CD19, resulting in stable antigen–antibody complex formation under the assay conditions. Other monoclonal antibodies directed against different B-cell or immune targets (such as CD20, CD3, PD-1/PD-L1, or unrelated antigens), or antibodies not specific to CD19, may exhibit reduced or minimal binding under these plate-bound conditions. This differential binding behaviour reflects the CD19 specificity of Blinatumomab as well as the controlled conformation and orientation of the immobilized CD19 antigen established during the immobilization process.



ELISA kits for Blinatumomab estimation offered by KRISHGEN uses Human CD19/Leu-12 capture proteins as above

3. Assay Validation

- IC50 Value: ~ 841.2 ng/ml (within 0-6000 ng/mL assay range).
- LLOQ: ~ 100.80 ng/ml.

- Clinical Cmax Values*:
 - Following the recommended dosing regimen (continuous intravenous infusion at 9 µg/day for the first week followed by 28 µg/day for subsequent weeks of the treatment cycle): ~0.5–1.5 ng/mL
 - Under steady-state conditions during continuous infusion: ~1–3 ng/mL

- Serum concentrations decline rapidly after infusion interruption due to the short elimination half-life (approximately 2 hours)

*Values are approximate and may vary depending on body weight, disease burden, treatment cycle, dosing schedule, organ function, and inter-patient variability.

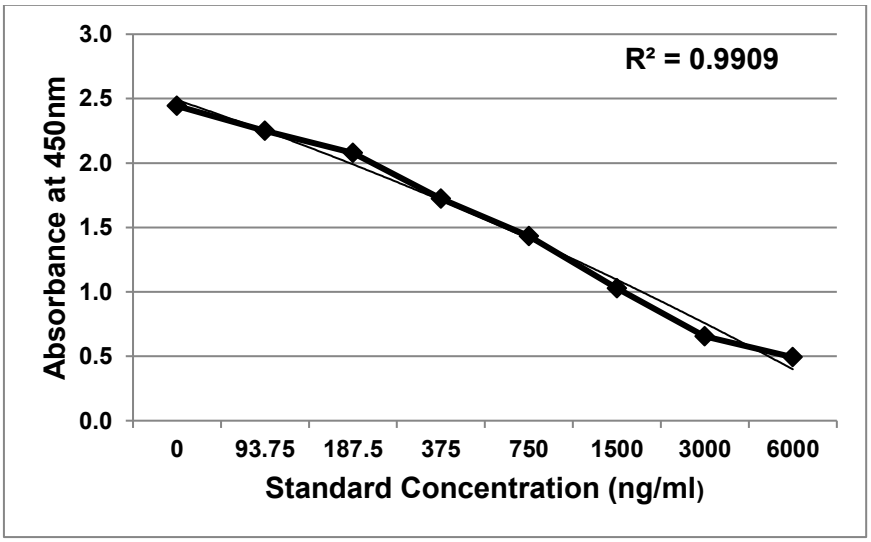
* *published data*

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

4. Standard Curve

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

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	%Interpolated Concentration against Actual Concentration
0	2.443	--	--
93.75	2.250	89.1	95.1
187.5	2.079	176.9	94.3
375	1.722	417.3	111.3
750	1.433	708.5	94.5
1500	1.027	1451.9	96.8
3000	0.654	3307.0	110.2
6000	0.493	5620.0	93.7



5. LOD and LOQ

- LOD Absorbance: (Approx ~93.89 ng/ml)
- LOQ Absorbance: (Approx ~100.80 ng/ml)

6. Pharmacokinetic Relevance

The assay is designed to cover the clinically relevant serum concentrations of Blinatumomab observed during continuous intravenous therapeutic administration, making it suitable for pharmacokinetic evaluation and therapeutic monitoring. The Blinatumomab ELISA demonstrates sensitivity within the ng/mL range, which aligns with the low circulating concentrations of this BiTE® molecule and ensures accurate quantification across clinically meaningful exposure levels.

Published pharmacokinetic data for Blinatumomab indicate systemic exposure consistent with continuous intravenous infusion of a short half-life therapeutic protein:

• Following the recommended dosing regimen (continuous intravenous infusion at 9 µg/day for the first week followed by 28 µg/day for subsequent weeks), steady-state serum concentrations typically range from approximately 0.5–3 ng/mL.

• Due to the short elimination half-life (approximately 2 hours), serum levels decline rapidly upon interruption or completion of the infusion.

• Exposure levels may vary depending on body weight, disease burden, treatment cycle, organ function, dosing schedule, and inter-patient variability.

Thus:

• At clinically relevant dosing conditions, Blinatumomab serum concentrations fall within the measurable range of the ELISA without the need for extensive sample dilution.

• The assay working range enables reliable differentiation across low systemic exposure levels.

• Minimal or no dilution of clinical samples is recommended, where appropriate, to maintain measurements within the linear dynamic range of the assay.

• The assay is therefore suitable for pharmacokinetic profiling, dose–exposure analysis, and therapeutic monitoring of Blinatumomab in human serum or plasma.

7. Precision and Reproducibility

Precision was assessed by analysing three standard concentrations (93.75 ng/ml, 750 ng/ml, and 6000 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
93.75	0.2% to 0.5%	<1%
750	0.8% to 1.4%	<2%
6000	2.1% to 4.5%	<5%

Observations:

- Intra-assay precision was consistently less than 4% across all levels tested.
- Inter-assay precision was consistently less than 5%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:
The KRIBIOLISA® Blinatumomab (BLINCYTO™) 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® Blinatumomab (BLINCYTO™) 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 30.09.2025