

ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH, BIOPHARMA
APPLICATIONS

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

Background

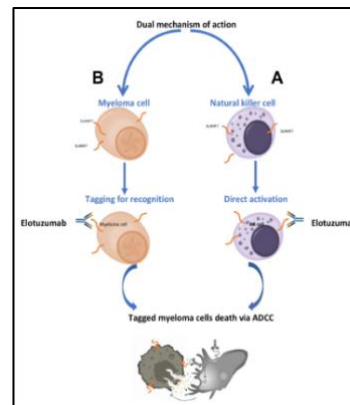
1. Introduction to Elotuzumab (EMPLICITI™)

Elotuzumab is a humanized monoclonal IgG1 antibody that specifically targets Signaling Lymphocytic Activation Molecule Family 7 (SLAMF7), a cell-surface glycoprotein highly expressed on multiple myeloma cells and natural killer (NK) cells. By binding to SLAMF7, Elotuzumab promotes direct activation of NK cells and facilitates antibody-dependent cellular cytotoxicity (ADCC) against SLAMF7-expressing malignant plasma cells. Elotuzumab also enhances NK-cell-mediated immune responses through engagement of SLAMF7 on immune effector cells, thereby strengthening antitumor activity within the tumor microenvironment. Clinically, Elotuzumab is used in combination with other anticancer therapies for the treatment of multiple myeloma, particularly in patients with relapsed or refractory disease. Its immune-mediated mechanism provides a targeted approach to eliminating malignant plasma cells while complementing the activity of immunomodulatory and proteasome inhibitor-based therapies.

2. Significance in Drug Discovery Research

2.1 Target Identification and Validation

- SLAMF7 (Signaling Lymphocytic Activation Molecule Family 7) is a cell-surface glycoprotein highly expressed on multiple myeloma cells and natural killer (NK) cells and plays an important role in immune-cell activation and malignant plasma-cell biology.
- Elotuzumab specifically binds to SLAMF7 on multiple myeloma cells and NK cells, enabling immune-mediated recognition and elimination of SLAMF7-expressing malignant plasma cells.
- Target validation studies can evaluate SLAMF7 expression, receptor density, antibody binding, and downstream NK-cell activation to establish the biological relevance of SLAMF7 as a therapeutic target.



2.2 Assay Development

- Elotuzumab serves as an important model for evaluating SLAMF7 target engagement, antibody binding, and immune-mediated cytotoxicity through:
 - ELISA – quantitative detection of Elotuzumab, assessment of antibody binding to recombinant SLAMF7, and characterization of antibody–target interactions.
 - Cell-based assays – evaluation of Elotuzumab binding to SLAMF7-positive multiple myeloma cells and assessment of NK-cell-mediated antibody-dependent cellular cytotoxicity (ADCC).
 - Flow cytometry-based assays – determination of SLAMF7 expression and receptor density on multiple myeloma cells and immune-cell populations.

- Immunoassays / multiplex biomarker panels – monitoring immune-response biomarkers, cytokines, and markers associated with NK-cell activation and antitumor activity.

2.3 Biomarker for Efficacy and Safety

- SLAMF7 expression, target engagement, NK-cell activation, immune-cell populations, and cytokine changes can be evaluated as biomarkers of pharmacodynamic response to Elotuzumab.
- Monitoring these immune and tumor-associated biomarkers can help characterize treatment response, immune activation, and potential immune-mediated adverse effects during preclinical and clinical development.
- Pharmacodynamic studies can evaluate SLAMF7 target engagement and immune-cell activation to support assessment of Elotuzumab exposure, biological activity, and dose-response relationships.

3. Relevance in Biopharmaceutical Development

3.1 Monoclonal Antibodies and Biosimilar Development

- Elotuzumab is a humanized IgG1 monoclonal antibody targeting SLAMF7 and is an important therapeutic antibody for the treatment of multiple myeloma.
- Development and characterization of Elotuzumab and potential biosimilars require evaluation of:
 - SLAMF7 binding assays
 - SLAMF7-positive cell binding assays
 - ADCC and cell-based functional assays
 - NK-cell activation assays
 - Comparability and analytical characterization studies

3.2 PK/PD Studies

- Elotuzumab serum concentrations are evaluated for pharmacokinetic (PK) parameters such as C_{max}, C_{min}, AUC, clearance, and half-life.
- Pharmacodynamic (PD) assessment can focus on SLAMF7 target engagement, receptor binding, NK-cell activation, and changes in immune or tumor-associated biomarkers.
- PK/PD studies help establish relationships between Elotuzumab exposure, target engagement, and antitumor activity, supporting dose selection and optimization.

3.2 Safety Evaluation

- Safety evaluation focuses on monitoring infusion-related reactions, immune activation, hematological abnormalities, infections, and other treatment-associated adverse events.

- Monitoring immune-cell populations, cytokine changes, and clinical biomarkers can help characterize the immune-mediated effects and safety profile of Elotuzumab during preclinical and clinical development.
- Combination therapy with immunomodulatory agents or other antimyeloma therapies requires additional assessment of cumulative immune effects and treatment-related toxicity.

4. Importance in Cell and Gene Therapy (CGT)

4.1 SLAMF7-Targeted Cell Therapy

- Elotuzumab targets SLAMF7, a highly expressed surface antigen on multiple myeloma cells, making the SLAMF7 pathway relevant to the development of targeted cellular immunotherapies.
- In CAR-T and CAR-NK cell therapies, SLAMF7 can serve as a potential tumor-associated target for engineered immune cells designed to recognize and eliminate SLAMF7-expressing malignant plasma cells.
- Elotuzumab can be used as a reference antibody or targeting tool for evaluating SLAMF7 expression, antigen density, and target specificity in engineered cell therapies.
- Studies can investigate whether SLAMF7-directed approaches enhance immune-cell activation, persistence, cytotoxicity, and antitumor activity against multiple myeloma cells.

4.2 Biomarker and Target Validation

- SLAMF7 expression and target engagement are important biomarkers for evaluating target availability and therapeutic activity in CGT studies.
- Elotuzumab can be evaluated in binding, target-engagement, flow cytometry, and functional cytotoxicity assays to characterize SLAMF7 expression and pathway-specific immune activity.
- Assessment of SLAMF7-positive tumor cells before and after treatment can help determine target-specific cell depletion and therapeutic response.

4.3 Genetic Modulation

- Genetic engineering approaches can be used to develop SLAMF7-directed CAR-T and CAR-NK cells for selective recognition of multiple myeloma cells.

Scope of Validation

This document presents a discussion of the characteristics of our KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA KIT (CATALOG NO. KBI1292) 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 Elotuzumab.

Validation characteristics considered by us in accordance with the guidelines are listed below:

- Specificity and Selectivity.
- Sensitivity (LOD & LOQ).
- Linearity and Range.
- Accuracy and Precision (Intra/Inter-Assay).
- Parallelism
- Matrix Effect.
- Disease state sample recovery

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 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.

Intended Use of the ELISA

To evaluate the specificity, assay performance, and clinical relevance of the KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA KIT, developed to quantitatively measure free, active Elotuzumab with high sensitivity and specificity.

Principle of the Assay

This ELISA is based on a sandwich immunoassay format. Recombinant SLAMF7 capture protein is immobilized on 96-well microplate wells. Elotuzumab present in the standards and test samples specifically binds to the coated antibodies during incubation. Following a wash step to remove unbound components, an Anti-Human IgG HRP-conjugated detection antibody is added, which binds to the captured Elotuzumab. After additional washing to eliminate excess conjugate, TMB substrate is added, allowing HRP to catalyze a colorimetric reaction. The reaction is then stopped by adding stop solution. The resulting yellow color is measured at 450 nm, where the optical density (OD) is directly proportional to the concentration of Elotuzumab present in the samples or standards.

Validation Parameters and Acceptance Criteria

1. Elotuzumab Values and Recommended ELISA Range

This table summarizes KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA KIT (Catalog No. KB11292) levels across different therapies and suggested corresponding ELISA working ranges.

Application	Expected Elotuzumab Range (ng/ml)	Recommended ELISA Range (ng/ml)
Post low-dose administration / early-phase treatment	10–100	0–150
Standard therapeutic dose / routine clinical treatment cycles	100–500	0–320
High-dose or combination therapy regimens	200–500	0–640
Pharmacokinetic monitoring / drug exposure–response evaluation	300–600	0–640

Note: Assay sensitivity of <5 ng/mL is recommended for baseline Elotuzumab detection, while an upper quantification limit of ≥250 ng/mL is desirable for therapeutic monitoring and assessment of higher Elotuzumab exposure in multiple myeloma patients.

The KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA Kit is developed using an assay range of 0–640 ng/mL, with dilution linearity and accuracy studies supporting the measurement of Elotuzumab responses across the concentrations relevant to the applications described above. The kit has also been validated up to 64,000-fold dilution, with measured values remaining within the acceptable accuracy and recovery criteria.

2. Specificity and Selectivity

2.1 Specificity

The capture and detection antibodies employed in the Elotuzumab ELISA are monoclonal antibodies that specifically recognize the intact humanized IgG1 therapeutic antibody Elotuzumab without cross-reacting with endogenous human immunoglobulins. These assay antibodies are selected to recognize unique idiotype or antibody-specific epitopes of Elotuzumab, ensuring high-affinity binding to the drug molecule in its native conformation. The specificity profile allows selective detection of Elotuzumab in complex biological matrices—such as serum, plasma, or cell-culture supernatants—while exhibiting minimal interference from structurally related therapeutic antibodies, SLAMF7, or unrelated immunoglobulins. This high degree of molecular discrimination enables accurate quantification of Elotuzumab, provided that the structural integrity of the target epitope is maintained.

2.2 Selectivity

The ELISA demonstrates minimal to no cross-reactivity with endogenous human IgG subclasses, recombinant antibodies, or structurally unrelated therapeutic monoclonal antibodies. It effectively excludes molecules that do not share the unique idiotype or antigenic determinants of Elotuzumab, including SLAMF7, soluble SLAMF7-related molecules, and other immune-cell surface proteins present in biological samples. The assay maintains high selectivity in complex biological matrices such as serum, plasma, or cell-culture supernatants, with negligible interference from matrix proteins, cytokines, heterophilic antibodies, Fc-binding serum factors, or other immunomodulatory molecules. This ensures reliable and accurate quantification of Elotuzumab without false-positive results arising from structurally related antibodies or immunologically active components.

2.3 LOD, LOQ and IC₅₀

LOD (Limit of Detection)

The lowest analyte concentration that can be reliably distinguished from blank/background noise but not necessarily quantified precisely.

Statistically:

LOD = Mean of Blank + 3X SD of Blank

(3σ criterion is most common).

LOD for KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA = 5.26 ng/ml

LOQ (Limit of Quantitation)

The lowest analyte concentration that can be quantified with acceptable accuracy and precision.

Statistically:

LOQ = Mean of Blank + 10X SD of Blank

(10σ criterion is most common).

The approximate LOQ for KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA – 10 ng/ml

IC₅₀ in ELISA (Half Maximal Inhibitory Concentration)

IC₅₀ = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

In ELISA, this is commonly used for:

Neutralization ELISA: Quantifies potency of antibodies inhibiting target–ligand interaction.

Drug Potency Testing: Measures concentration at which drug inhibits 50% of target activity.

IC₅₀ for KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA = ~163.6 ng/ml

Summary:

Parameter	Value (ng/ml)
LOD	5.26 ng/ml
LOQ	10 ng/ml
IC ₅₀	163.6 ng/ml

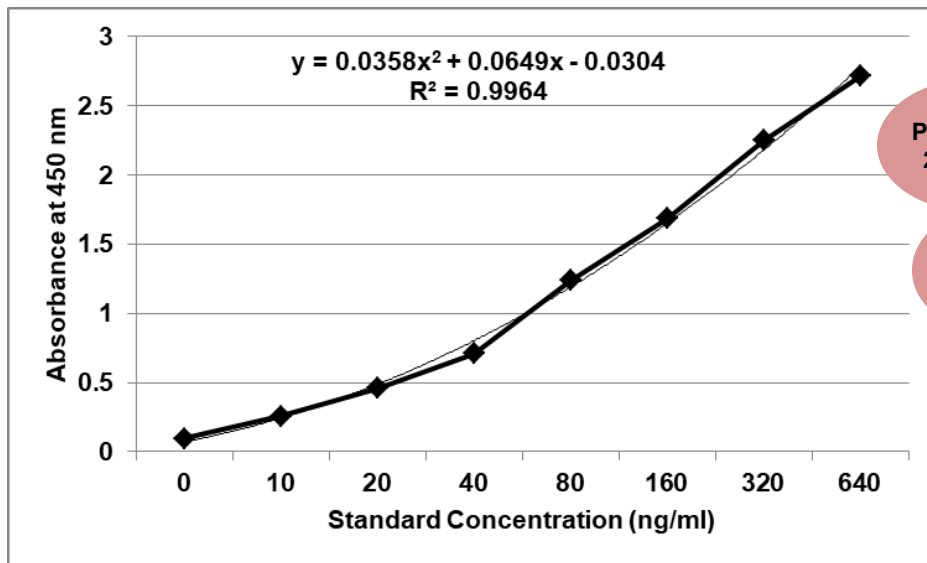


Regulatory Note:

LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% *S/N = Signal / Noise Ratio

3. Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
0	0.094	--	--
10	0.256	10.2	102.0
20	0.456	20.5	102.6
40	0.707	36.6	91.6
80	1.238	86.3	107.8
160	1.687	155.3	97.0
320	2.249	319.5	99.8
640	2.716	643.6	100.6
Low QC (10 ng/ml)	0.249	8.5	84.7
Mid QC (160 ng/ml)	1.658	149.0	93.1
High QC (600 ng/ml)	2.584	517.5	86.2



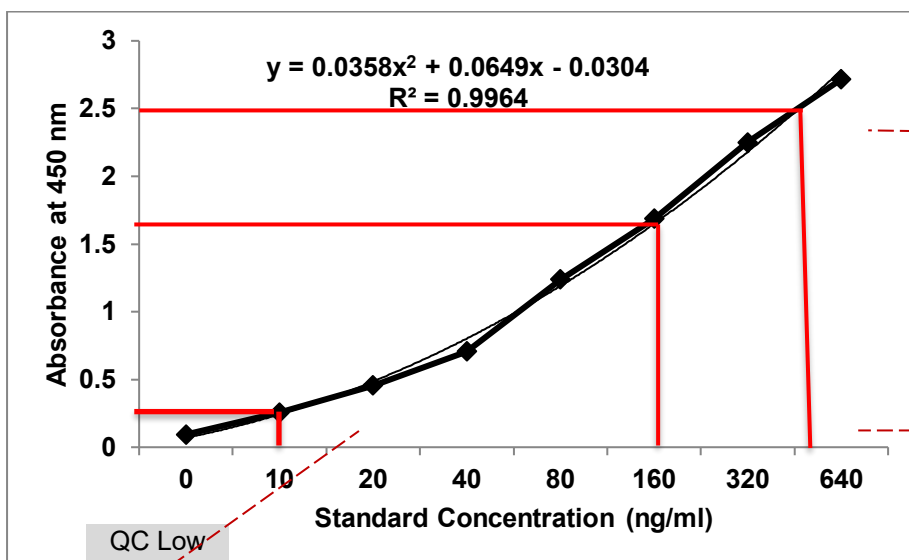
Polynomial
2nd Order

$R^2 =$
99.64%

Accuracy
%

Intra
CV%

Inter
CV%



QC High

86.2%

4.2% to
5%

<3.6%

QC Mid

93.1%

4.2% to
5%

<3.6%

QC Low

84.7%

0.5% to
1.3%

<3.5%

Figure 1. Standard Curve Accuracy — % Recovery Across the Assay Range

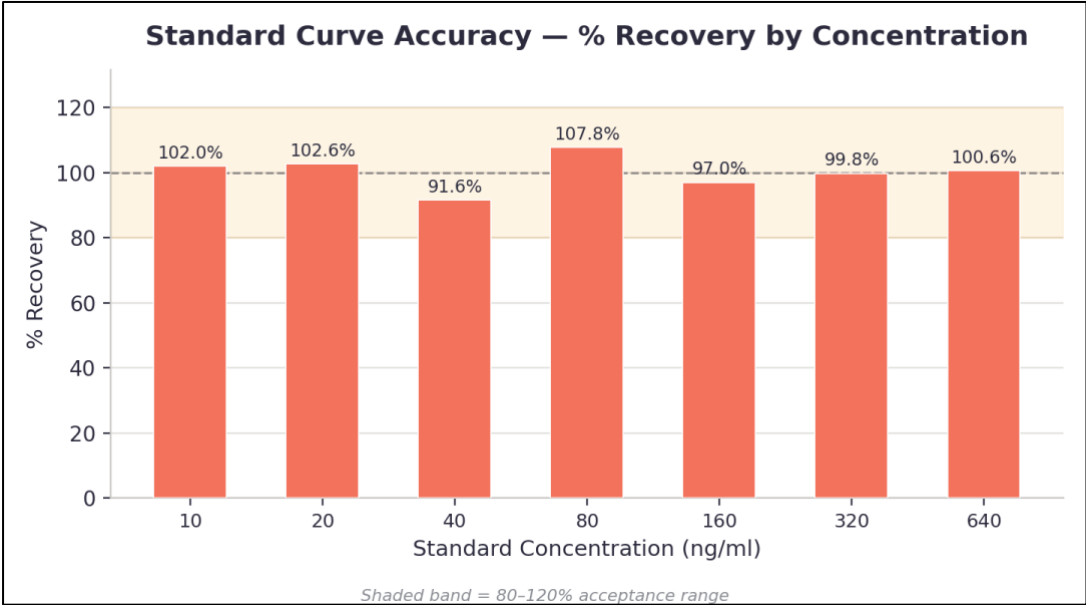
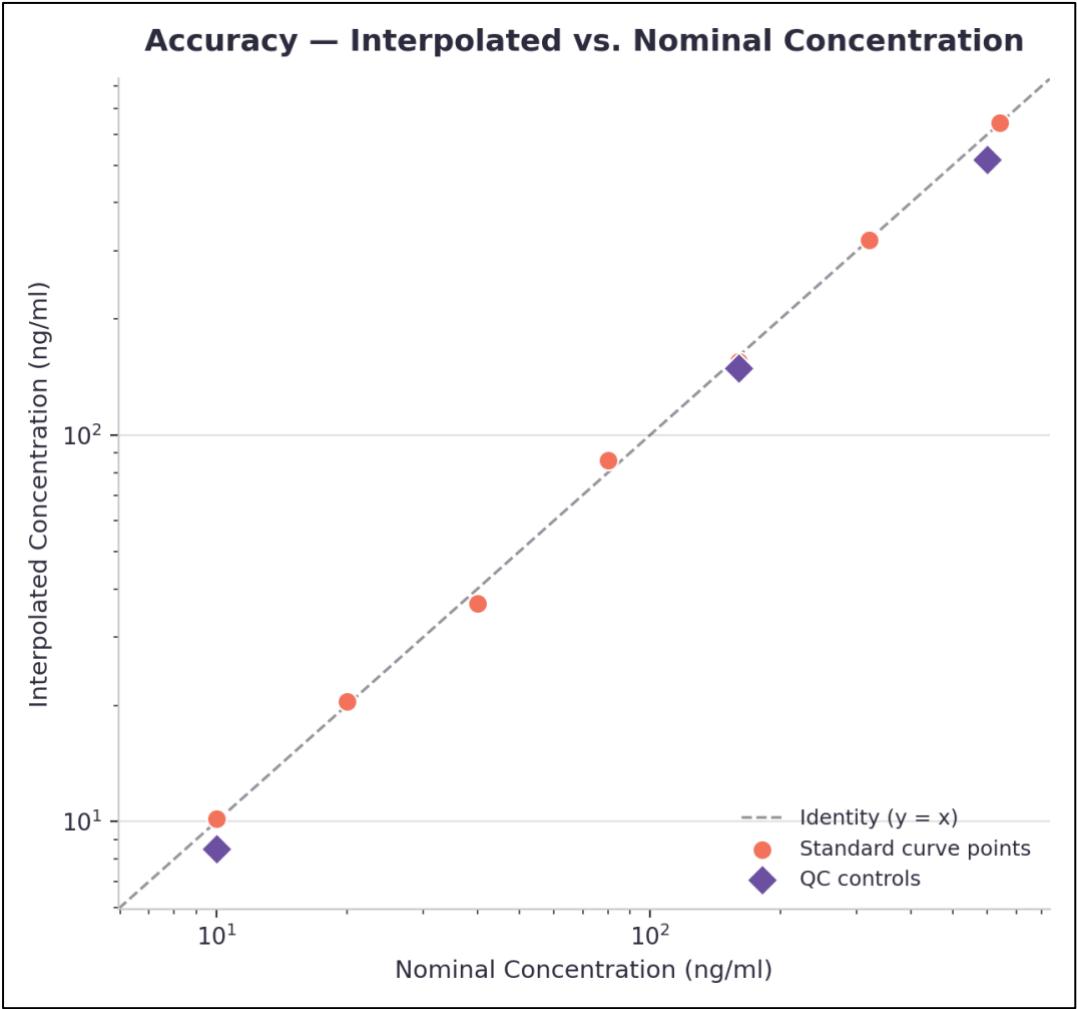


Figure 2. Accuracy — Interpolated vs. Nominal Concentration (Log-Log)



4. Accuracy and Precision (Intra / Inter-Assay)

A) Intra-Assay:

Standard Concentration (ng/ml)	Mean OD450	SD	%CV
10	0.245	0.04	4.5
80	1.228	0.08	4.3
640	2.704	0.04	1.9

B) Inter Assay:

Standard Concentration (ng/ml)	Mean OD450	SD	%CV
10	0.238	0.05	3.2
160	1.224	0.02	4.2
640	2.695	0.04	2.1

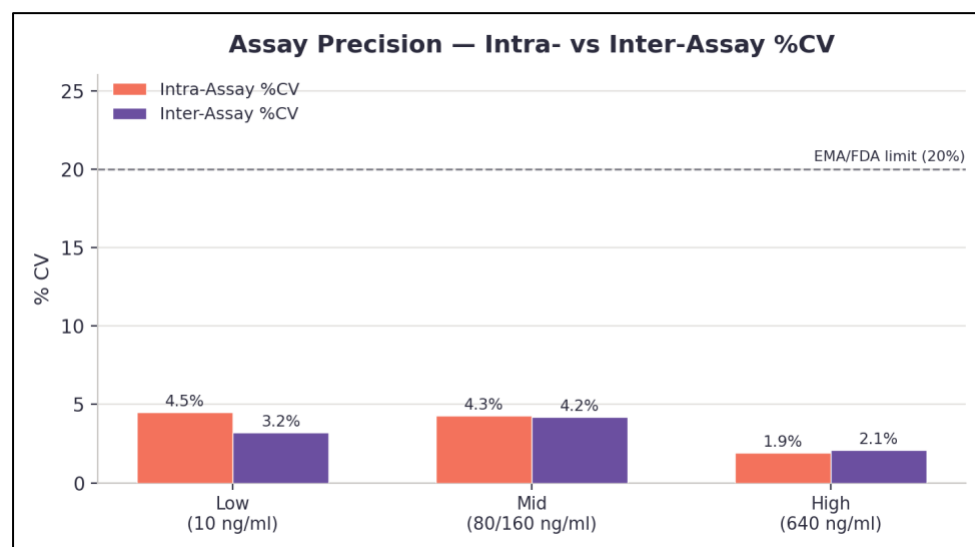
Observations:

- Intra-assay precision was consistently less than 4.6% across all levels tested.
- Inter-assay precision was consistently less than 4.4%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:

The KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA demonstrates excellent intra- and inter-assay precision. These results support the assay's reliability and reproducibility for routine use in pharmacokinetic and bio analytical studies.

Figure 3. Intra- vs Inter-Assay Precision (%CV)



5. Parallelism

Serial dilutions of a high-concentration sample were prepared at dilutions of 1:1, 1:2, 1:4, 1:8, 1:16 and 1:32 for both human serum and human plasma. Each dilution was assayed using the KRIBIOLISA® Elotuzumab (EMPLICITI™) ELISA and compared to the standard curve.

Acceptance Criteria:

- The back-calculated concentration (interpolated) should fall within $\pm 20\%$ of the expected concentration across the tested range.
- % Recovery should be between 80% and 120% for most samples.

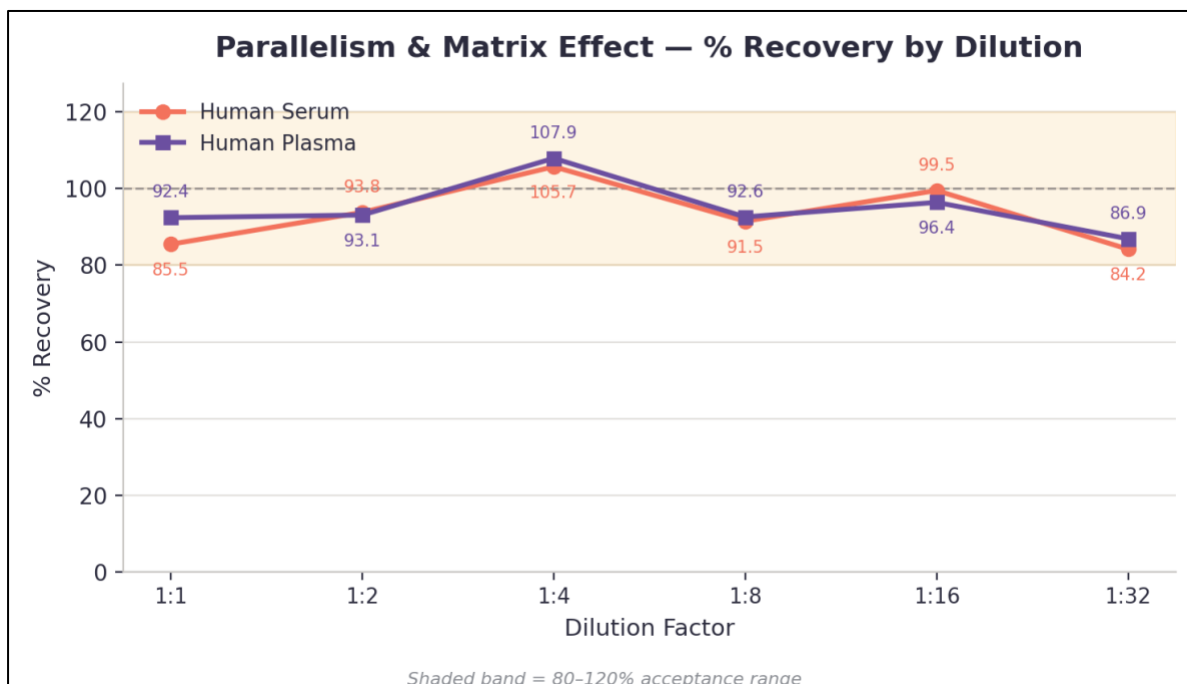
A) Human Serum:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	320	2.145	273.6	85.5	101.8
1:2	160	1.664	150.1	93.8	92.6
1:4	80	1.214	84.54	105.7	93.0
1:8	40	0.695	36.5	91.5	97.1
1:16	20	0.448	19.9	99.5	97.8
1:32	10	0.248	8.4	84.2	89.1

B) Human Plasma:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	320	2.204	295.82	92.4	95.5
1:2	160	1.658	149.00	93.1	92.4
1:4	80	1.229	86.28	107.9	99.7
1:8	40	0.701	37.04	92.6	102.9
1:16	20	0.438	19.29	96.4	101.5
1:32	10	0.253	8.69	86.9	118.7

Figure 4. Parallelism & Matrix Effect — % Recovery by Dilution (Serum vs. Plasma)



Results:

- Parallelism is generally maintained across the 1:1 to 1:32 dilutions.
- % Recovery for all dilutions falls within the acceptable range of 80–120%.
- No significant matrix effect observed at higher dilutions.
- The KRIBOLISA® Elotuzumab (EMPLICITI™) ELISA kit was tested for matrix effect on human serum and plasma.

Conclusion:

Parallelism was demonstrated between the diluted samples and the standard curve. This supports the validity of using sample dilutions within the working range of the Elotuzumab (EMPLICITI™) ELISA without significant loss of accuracy.

6. Matrix Effect Study

Matrix effect was evaluated by comparing the assay performance of standards prepared in:

- Assay buffer (only buffer)
- Assay buffer spiked with human serum (buffer + 1:1000 human serum)
- Assay buffer spiked with human serum (buffer + 1:1000 human plasma)

Samples were tested across the standard curve range (0–640 ng/ml). Mean absorbance, % Standard Deviation, and % Coefficient of Variation (%CV) were calculated to assess the impact of the serum matrix.

Matrix Effect Study Results

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:1000 Human Serum)	% Standard Deviation	% CV
0	0.105	0.108	0.21	2.0
10	0.245	0.239	0.93	1.8
20	0.448	0.426	1.38	3.6
40	0.698	0.652	0.12	4.8
80	1.225	1.256	4.07	1.8
160	1.648	1.592	6.89	2.4
320	2.195	2.135	4.58	2.0
640	2.769	2.854	1.27	2.1

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:1000 Human Plasma)	% Standard Deviation	% CV
0	0.105	0.115	0.71	6.4
10	0.245	0.235	0.93	2.9
20	0.448	0.425	1.38	3.7
40	0.698	0.752	0.12	5.3
80	1.225	1.254	4.07	1.7
160	1.648	1.758	6.89	4.6
320	2.195	2.051	4.58	4.8
640	2.769	2.692	1.27	2.0

Results:

- Very low %CV across all concentrations.
- Minimal shift in absorbance values between buffer-only and buffer + serum and buffer + plasma conditions.
- No significant matrix effect observed

6. Disease State Sample recovery

Sample ID	Disease Type	Patient details Ethnicity Age/Sex	Diabetic/ Smoker	Pathogen	Medi- cation	Blood Type/ BMI	Anticoa- gulant
110026 298	Multiple Myeloma	Caucasian 62/Female	No/No	Negative	Nil	Nil	Nil

Recovery was assessed by analyzing diseased serum samples (n=1) across multiple dilution factors (1:2 to 1:32) against calibration standards. This study was conducted to evaluate potential matrix effects and the ability of the assay to accurately recover Elotuzumab in clinically relevant diseased serum matrices.

Sample Details:

Diseased-state sample validation was performed using one clinically relevant human serum samples from patients with multiple myeloma. The samples included female subject, aged 62 years, and the sample was of Caucasian demographic. The sample was reported as non-diabetic and pathogen-negative. The sample was evaluated to assess the assay performance and suitability in a clinically relevant diseased-state matrix.

Acceptance Criteria:

- Recovery within 70–130% is generally considered acceptable for ligand-binding assays.
- Consistency across dilutions indicates minimal matrix interference.

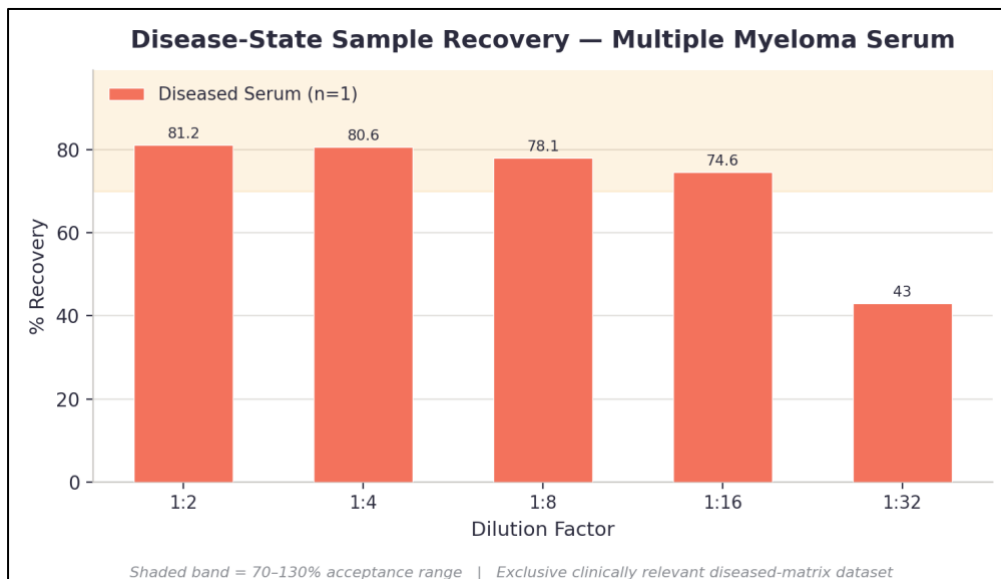
Diseased State Sample Impact on Elotuzumab Recovery

Sample Matrix	1:2 Dilution (%)	1:4 Dilution (%)	1:8 Dilution (%)	1:16 Dilution (%)	1:32 Dilution (%)
Diseased Serum (n=1)	81.2	80.6	78.1	74.6	43

Observations:

- Recoveries remained relatively consistent across the 1:2 to 1:16 dilution range (74.6–81.2%), indicating good analyte detectability and minimal impact of matrix interference under these conditions.
- A gradual reduction in recovery was observed with increasing dilution, which is expected due to decreasing analyte concentration and potential dilution-related effects.
- Recovery at the 1:32 dilution decreased to 43%, suggesting that this dilution exceeds the assay's optimal working range for accurate quantification in diseased serum.
- The results indicate that the assay can reliably measure Elotuzumab in diseased serum samples when tested at dilution factors up to 1:16

Figure 5. Disease-State Sample Recovery — Diseased Serum (Multiple Myeloma)



9. Sample Handling and Storage Conditions

A.) Sample collection and handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at temperature $<-20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

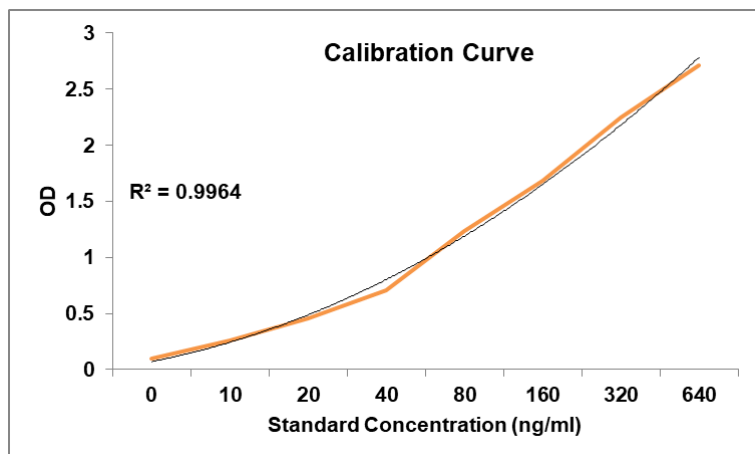
Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at $1000 \times g$. Remove serum layer and assay immediately or store serum samples at temperature $<-20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at $1000 \times g$ within 30 minutes of collection. Assay immediately or store plasma samples at temperature $<-20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

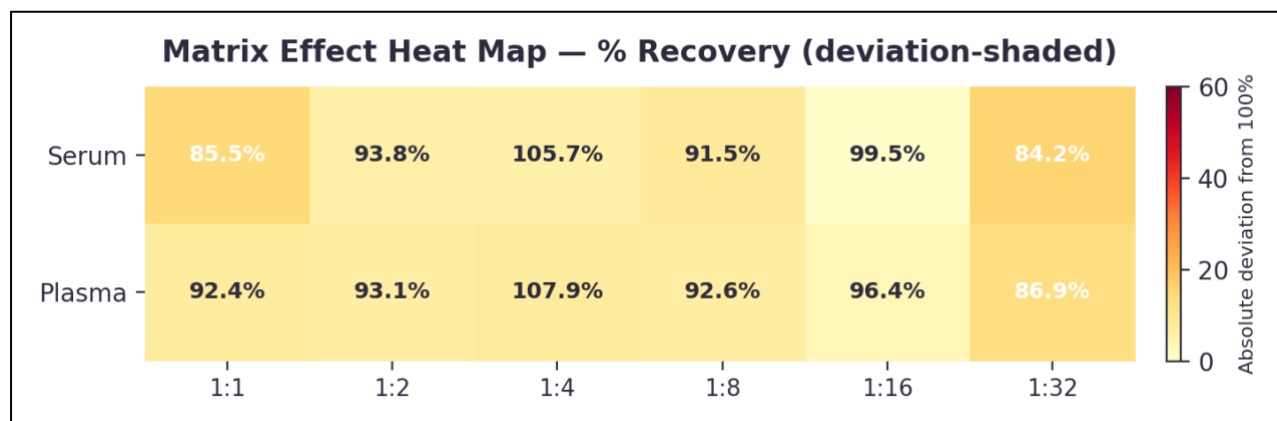
B) Storage conditions:

- It is advisable to aliquot and store the Anti-Pembrolizumab:HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at $2-8^{\circ}\text{C}$. Immediately discard any excess Working Anti-Pembrolizumab:HRP Conjugate after running your assay.
- All the reagents and wash solutions should be used within 12 months from manufacturing date.
- Before using, bring all components to room temperature ($18-25^{\circ}\text{C}$). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
- The Substrate is light-sensitive and should be protected from direct sunlight or UV source

Graphs, Maps and Appendices:



Matrix Effect Heat Map



	1:1	1:2	1:4	1:8	1:16	1:32
Serum						
Plasma						

Determined Limits for Acceptance according to EMA/FDA and CLSI regulations

	Limits for Acceptance (EMA/FDA)	Determined Limits for Acceptance (CLSI)
Intra Precision	CV < 20% (25% at LLOQ)	-
Inter Precision	CV < 20 % (25% at LLOQ)	-
Accuracy at LLOQ	Recovery 100 ± 20%	-
Total Error (TE)	TE < 30% (40% at LLOQ and ULOQ)	-
Specificity/Interference	Recovery 100 ± 25%	H (null hypothesis) = 100 ± 25 %
Parallelism/Linearity	CV < 30%	Deviation from linearity < 20%
LLOQ / LOQ	Recovery 100 ± 25%	TE % < 32.9%

References

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