

ELISA VALIDATION GUIDE

ASSAY FOR USE IN

DRUG DISCOVERY RESEARCH,
BIOPHARMA APPLICATIONS

KRISHGEN *BioSystems*

OUR REAGENTS, YOUR RESEARCH

Background

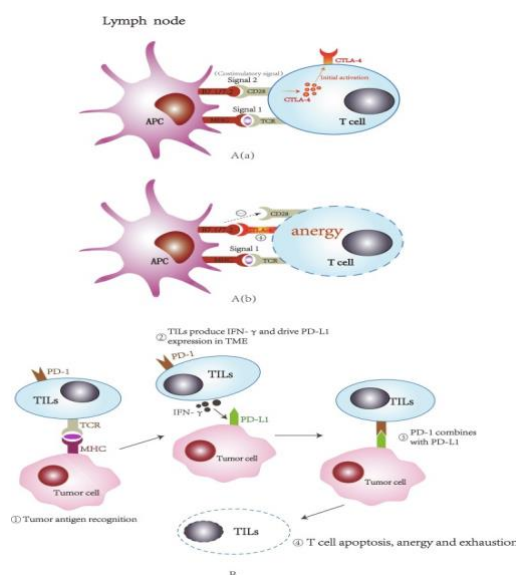
1. Introduction to Camrelizumab

Camrelizumab is a humanized monoclonal antibody that targets the programmed cell death protein 1 (PD-1) receptor, an important immune checkpoint involved in the regulation of T-cell activity. By binding to PD-1 and blocking its interaction with its ligands, PD-L1 and PD-L2, Camrelizumab helps restore T-cell-mediated immune responses against tumor cells. Camrelizumab is used in the treatment of various malignancies, including certain types of lung cancer, liver cancer, esophageal cancer, and other solid tumors. As an immune checkpoint inhibitor, it has emerged as an important therapeutic agent in modern oncology and immuno-oncology drug discovery and development programs.

2. Significance in Drug Discovery Research

2.1 Target Identification and Validation

- Camrelizumab targets the programmed cell death protein 1 (PD-1) receptor, an important immune checkpoint involved in the regulation of T-cell activity and immune responses.
- The PD-1 signaling pathway plays an important role in tumor immune evasion, as interactions between PD-1 and its ligands, PD-L1 and PD-L2, can suppress T-cell-mediated immune responses.
- Blocking the PD-1/PD-L1 signaling pathway represents an important therapeutic strategy for restoring antitumor immune activity and improving immune-mediated recognition and elimination of tumor cells.



2.2 Assay Development

- Camrelizumab can be evaluated during drug discovery and development using various analytical and functional assays, including:
 - ELISA for the quantification of Camrelizumab in biological samples or the assessment of relevant biomarkers.
 - Cell-based assays for evaluating the biological activity of Camrelizumab and its ability to inhibit PD-1-mediated immune checkpoint signaling.
 - Binding assays for assessing the interaction of Camrelizumab with the PD-1 receptor.
 - Pharmacokinetic and immunogenicity assays for monitoring drug concentration, exposure, and potential anti-drug antibody responses.

2.3 Biomarkers for Efficacy and Safety

- Camrelizumab-related biomarkers may be used to evaluate target engagement, pharmacodynamic activity, and therapeutic response.
- Evaluation of PD-1 pathway-related biomarkers, including PD-L1 expression where relevant, may support the assessment of treatment response and immune activity.
- Pharmacokinetic, immunogenicity, and safety assessments are important components of the preclinical and clinical development of Camrelizumab.
- Monitoring immune-related effects and treatment-associated adverse events is important when evaluating the overall safety profile of Camrelizumab.

3. Relevance in Biopharmaceutical Development

3.1 Monoclonal Antibodies and Biosimilar Development

- Camrelizumab is a humanized monoclonal antibody that targets the programmed cell death protein 1 (PD-1) receptor.
- The development and characterization of therapeutic monoclonal antibodies and potential biosimilar products require comprehensive analytical, physicochemical, and functional evaluation, including:
 - Binding assays to assess affinity and interaction with the PD-1 receptor.
 - Cell-based potency assays to evaluate the biological activity of the antibody and its ability to block PD-1-mediated signalling.
 - Physicochemical and biological comparability studies to demonstrate consistency between different product batches or candidate products.
 - Pharmacokinetic and immunogenicity assessments as part of product development and regulatory evaluation.

3.2 PK/PD Studies

- Camrelizumab concentrations may be monitored during preclinical and clinical studies to characterize its pharmacokinetic profile.
- Pharmacodynamic assessments may include the evaluation of target engagement and changes in biomarkers associated with PD-1 pathway modulation and immune activation.
- PK/PD studies can help establish the relationship between drug exposure, biological activity, therapeutic response, and safety.

3.3 Safety Evaluation

- The safety of Camrelizumab is evaluated throughout preclinical and clinical development using appropriate safety and tolerability assessments.
- Monitoring of treatment-related adverse effects, including immune-related adverse events, immunogenicity, and other clinically relevant safety parameters, is important during therapeutic development.
- Appropriate analytical methods for the quantification of Camrelizumab and the assessment of relevant pharmacodynamic and immune-related biomarkers can support pharmacokinetic, pharmacodynamic, efficacy, and safety evaluations.

4. Importance in Cell and Gene Therapy (CGT)

4.1 Therapeutic Development and Safety Monitoring

- Camrelizumab is an immune checkpoint inhibitor targeting the programmed cell death protein 1 (PD-1) receptor and is not a primary cytokine mediator of cytokine release syndrome (CRS).
- However, appropriate safety monitoring is important when evaluating Camrelizumab in combination with cellular or gene-based therapeutic approaches.
- The assessment of treatment-related immune responses, immune activation, adverse events, and other relevant safety parameters is important during preclinical and clinical development.

4.2 Target Engagement and Biomarker Evaluation

- Camrelizumab-related studies may be relevant for evaluating PD-1 expression and PD-1 pathway activity in cellular models used for therapeutic research.
- The assessment of PD-1 pathway-related biomarkers, including PD-L1 and PD-L2 where relevant, may support the evaluation of target engagement, biological activity, and therapeutic response.
- Quantification of Camrelizumab may also support pharmacokinetic and pharmacodynamic studies during therapeutic development.

4.3 Genetic and Cellular Engineering Research

- Genetic and cellular engineering approaches may be used to develop experimental models with altered PD-1 or PD-L1 pathway expression to study the mechanism of action and biological activity of Camrelizumab.
- Such models can support the investigation of immune checkpoint regulation, treatment response, and potential mechanisms of therapeutic resistance.
- Cellular models may also be used to investigate the effects of PD-1 pathway modulation on immune cell activation and tumor cell recognition.
- These studies may contribute to the development of personalized therapeutic strategies and the identification of patient populations that may benefit from PD-1-targeted immunotherapies.

Scope of Validation

The KRIBIOLISA® Camrelizumab ELISA Kit (Catalog No. KBI1214) has been considered by us during the validation of this assay in accordance with applicable FDA/ICH guidelines for bioanalytical method validation. This document is prepared based on tests performed in our laboratory and does not necessarily seek to cover all testing that may be required at the user's end for registration or regulatory submissions. The objective of this validation is to demonstrate that the assay is suitable for its intended purpose—the detection and quantification of Camrelizumab in appropriate biological samples.

The validation characteristics considered by us are listed below:

- Assay Validation.
- Standard Curve.
- Pharmacokinetic Relevance.
- Precision and Reproducibility.
- Parallelism.
- Disease-State Sample Recovery.
- Matrix Effect.

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 the user's laboratory. This data has been generated to enable the user to obtain an overview of the assay and its performance characteristics and is generic in nature. We recommend that the user performs, at a minimum, a spike and recovery assay to assure quality results.

For more comprehensive validation, the user may perform additional studies as appropriate to establish quality control parameters for the intended application.

For any queries or support regarding the data and its performance, please contact us at sales1@krishgen.com.

Intended Use of the ELISA

The KRIBIOLISA® Camrelizumab ELISA Kit is intended for the quantitative measurement of Camrelizumab in appropriate biological samples, including human serum and plasma, for research, pharmacokinetic, and bioanalytical applications.

Principle of the Assay

This ELISA is a sandwich immunoassay. Antibodies are coated on 96 well plates. The antigen protein present in sample and standard respectively bind to the coated wells. The wells are washed and an antibody:HRP Conjugate is added which binds to the bound complex in the well. Washing is performed to remove any unbound material. TMB substrate is added and the enzyme reaction is stopped by dispensing of stop solution into the wells. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of antigen protein present in the standard or samples.

Validation Parameters and Acceptance Criteria

1. Camrelizumab Pharmacokinetic Relevance and Recommended ELISA Range

The following section summarizes the pharmacokinetic relevance of Camrelizumab and the recommended working range of the KRIBIOLISA® Camrelizumab ELISA Kit (Catalog No. KBI1214).

Application	Expected Camrelizumab Concentration	Recommended ELISA Working Range
Bioanalytical and Pharmacokinetic Studies	Concentrations may vary depending on tumour type, body weight, disease status, dosing schedule, combination therapy, and inter-patient variability.	0–640 ng/ml
Initial Therapeutic Exposure	Approximately 40–70 µg/ml following the recommended intravenous dosing regimen.	Appropriate sample dilution recommended before analysis.
Steady-State Exposure	Approximately 60–100 µg/ml following multiple treatment cycles.	Appropriate sample dilution recommended before analysis.
Trough Concentration	Approximately 15–35 µg/ml prior to the next dose at steady state.	Appropriate sample dilution recommended before analysis.
Therapeutic Monitoring and Exposure Assessment	Variable depending on tumour type, dosing regimen, body weight, and individual patient variability.	Samples should be diluted, where necessary, to fall within the validated assay range.

Note: The KRIBIOLISA® Camrelizumab ELISA Kit has an assay concentration range of 0–640 ng/ml. Due to the relatively high circulating concentrations of Camrelizumab following therapeutic administration, clinical serum or plasma samples may require appropriate dilution before analysis to ensure that the measured concentration falls within the linear dynamic range of the assay.

The assay demonstrates measurable sensitivity within the working range of approximately 10–640 ng/ml, with an approximate LOQ/LLOQ of 10 ng/ml. The assay is therefore suitable for pharmacokinetic profiling, dose–exposure analysis, and therapeutic monitoring following appropriate sample dilution.

2. Specificity and Selectivity

2.1 Specificity

The KRIBIOLISA® Camrelizumab ELISA employs a targeted ligand-binding approach using Recombinant Human PD1/PDCD1 Protein as the capture protein to facilitate the selective detection of Camrelizumab. Camrelizumab is a humanized IgG4 monoclonal antibody that specifically targets the programmed cell death protein-1 (PD-1) receptor. The assay is designed to support selective interaction between Camrelizumab and the immobilized PD-1 capture protein.

2.2 Selectivity

The ELISA shows no or low cross-reactivity with other immune checkpoint inhibitors, such as nivolumab and pembrolizumab.

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).

The approximate LOD for the KRIBIOLISA® Camrelizumab ELISA is 5.85 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/LLOQ for the KRIBIOLISA® Camrelizumab ELISA is 10 ng/ml
IC₅₀ in ELISA (Half Maximal Inhibitory Concentration)

For the KRIBIOLISA® Camrelizumab ELISA validation, the observed IC₅₀ value is 185 ng/ml

Summary:

Parameter	Value (ng/ml)
LOD	~5.85 ng/ml
LOQ	~10 ng/ml
IC ₅₀	~185 ng/ml



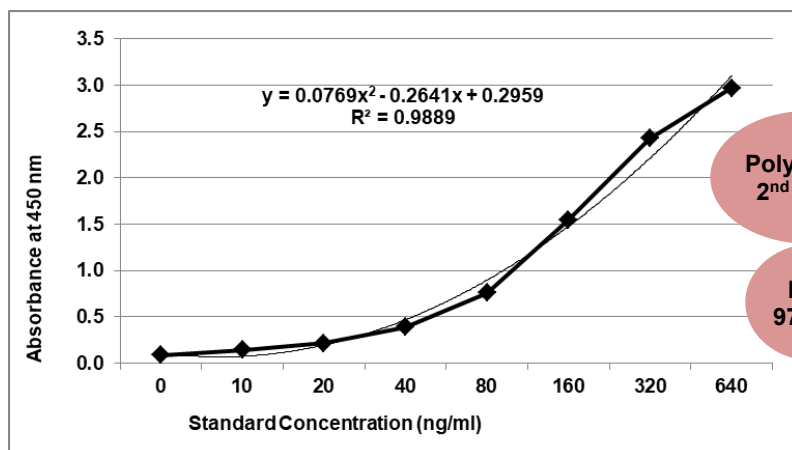
Regulatory Note:

LOD *S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20%

*S/N = Signal / Noise Ratio

2. Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
0	0.090	--	--
10	0.147	11.0	110.3
20	0.216	22.1	110.6
40	0.393	42.6	106.6
80	0.759	77.8	97.3
160	1.548	159.8	99.9
320	2.425	323.7	101.2
640	2.970	632.4	98.8
Low QC (20 ng/ml)	0.214	23.9	119.5
Mid QC (500 ng/ml)	2.474	548.5	109.7
High QC (600 ng/ml)	2.705	693.3	115.6



Polynomial
2nd Order

R² =
97.53%

Accuracy
%

Intra
CV%

Inter
CV%

QC Medium

109.7%

2.3%

1%

QC High

115.6%

1.6%

2.1%

QC Low

119.5%

5.1%

2.2%

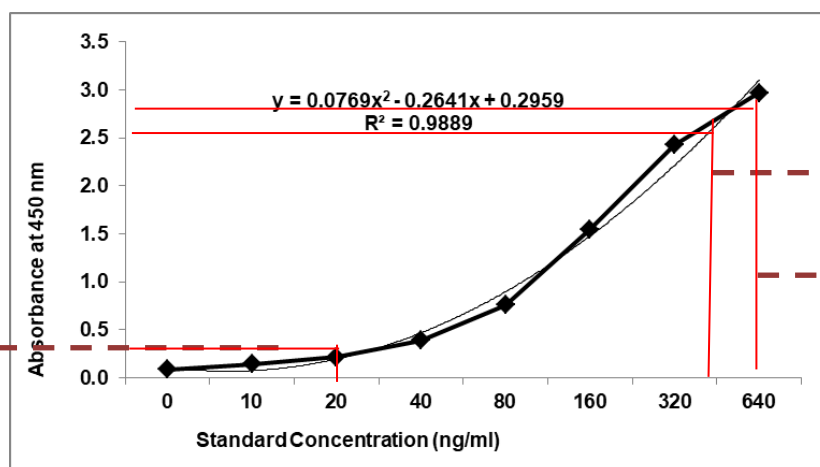


Figure 1. Standard Curve Accuracy — % Recovery Across the Assay Range

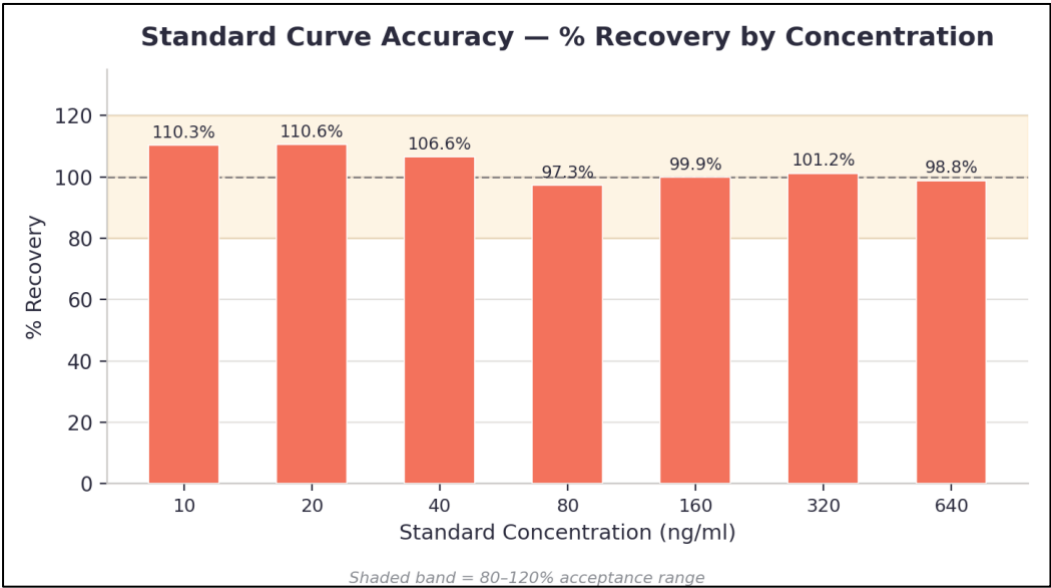
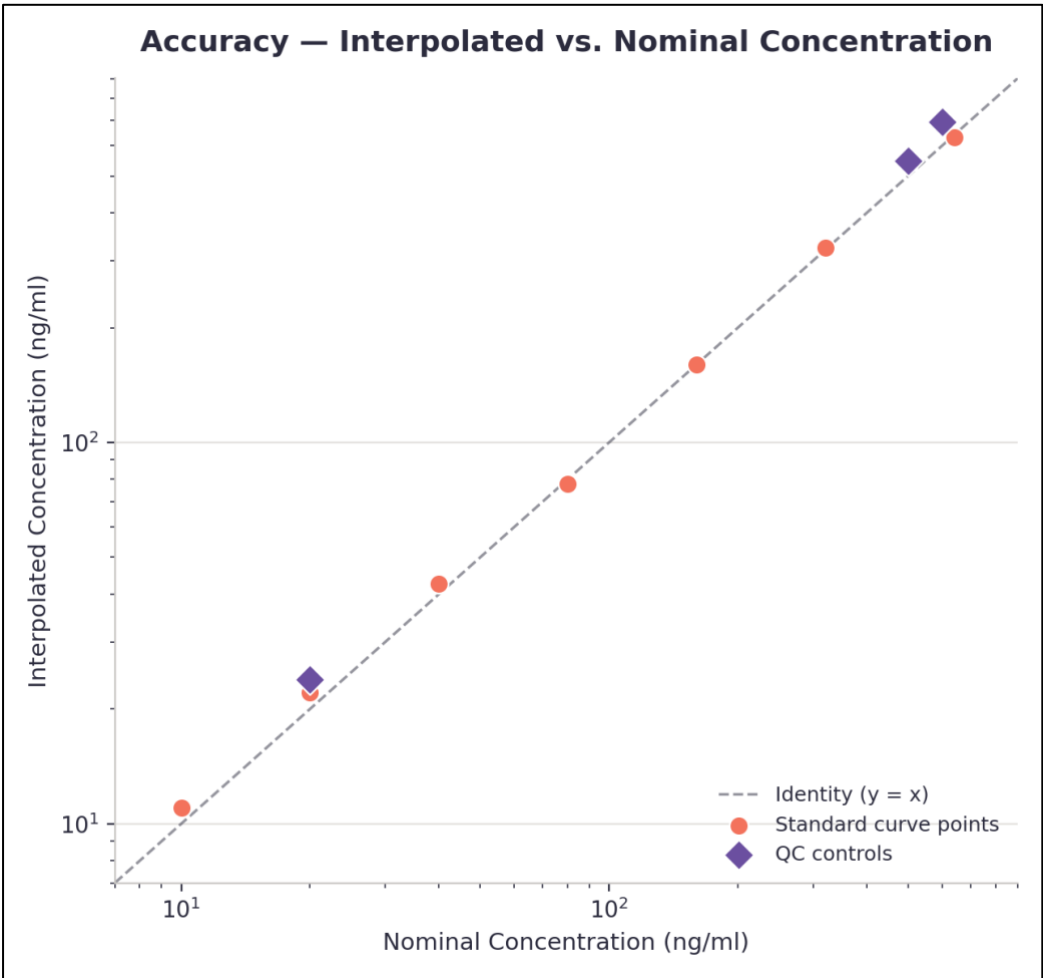


Figure 2. Accuracy — Interpolated vs. Nominal Concentration (Log-Log)



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

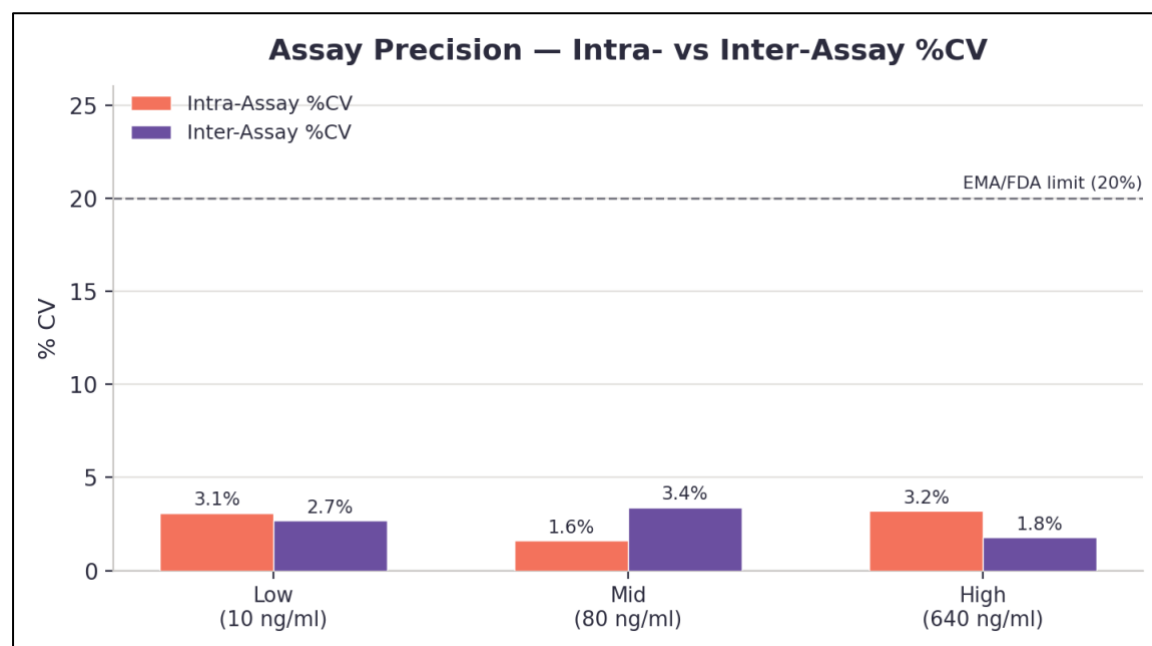
A) Intra-Assay:

Standard Concentration (ng/ml)	Mean OD450	SD	%CV
10	0.139	0.0	3.1
80	0.742	0.0	1.6
640	2.895	0.1	3.2

B) Inter Assay:

Standard Concentration (ng/ml)	Mean OD450	SD	%CV
10	0.145	0.00	2.7
80	0.725	0.03	3.4
640	2.906	0.05	1.8

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



4. Parallelism and Matrix Effect

Sample Dilution factor – Human Serum and Human Plasma samples have been tested.

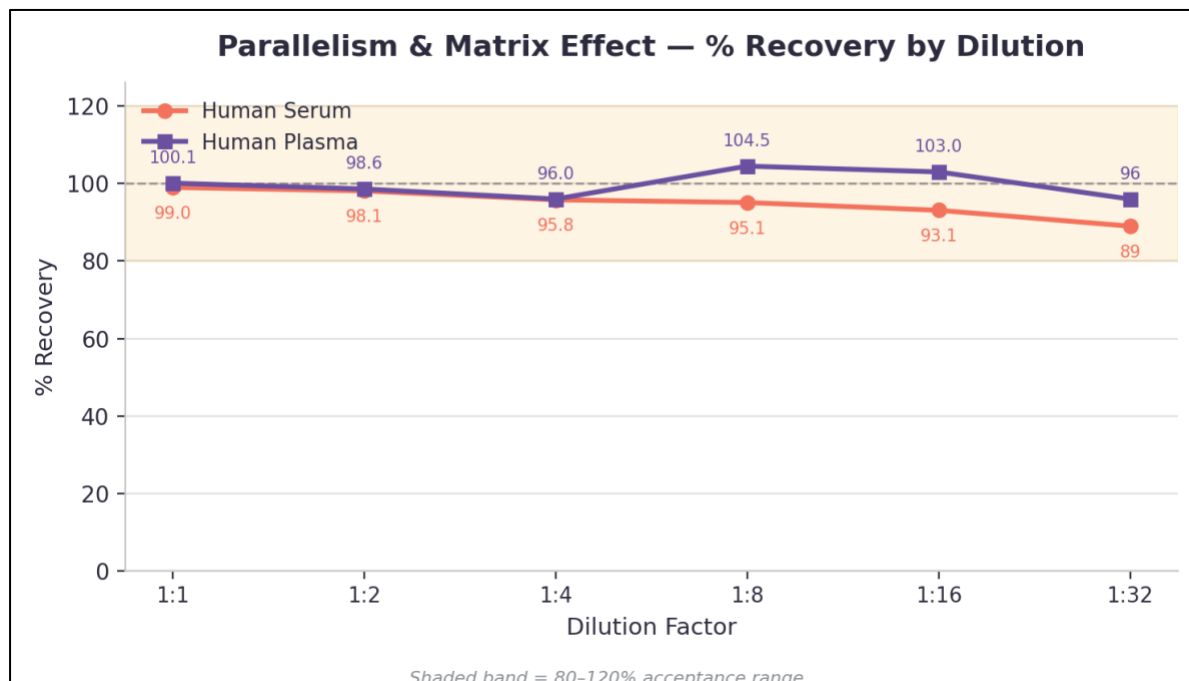
A) Serum:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	320	2.402	316.8	99.0	100.6
1:2	160	1.525	157.0	98.1	103.4
1:4	80	0.745	76.6	95.8	98.8
1:8	40	0.305	33.0	95.1	105.2
1:16	20	0.205	20.5	93.1	107.4
1:32	10	0.139	8.9	89	93.4

B) Plasma:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	320	2.415	320.6	100.1	100.4
1:2	160	1.532	157.9	98.6	112.4
1:4	80	0.748	76.8	96.0	103.9
1:8	40	0.385	41.8	104.5	78.2
1:16	20	0.206	20.6	103.0	87.1
1:32	10	0.142	9.6	96	110.4

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



Results:

- i) Parallelism is maintained across the 1:1 to 1:32 dilutions.
- ii) % Recovery for most dilutions falls within the acceptable range of 80%–120%.
- iii) No significant matrix effect observed at higher dilutions.
- iv) The KRIBIOLISA® Camrelizumab ELISA kit was tested for matrix effect on human serum and human plasma samples.

5. Disease State Sample Recovery

Sample Details:

Diseased-state sample validation was performed using six clinically relevant human samples, comprising four NSCLC human plasma samples, one Chronic Myelogenous Leukemia (CML) human serum and Chronic Myelogenous Leukemia (CML) human plasma sample. The samples included both male and female subjects, with ages ranging from 56 to 68 years, and represented Hispanic and Caucasian demographics. All samples were reported as non-diabetic and pathogen-negative. These clinically relevant diseased matrices were evaluated to assess the assay's performance and suitability for detecting the target analyte in disease-state samples.

Sample ID	Disease Type	Patient details Ethnicity Age/Sex	Diabetic/ Smoker	Pathogen	Medi- cation	Blood Type/ BMI	Anticoa- gulant
PL1022 19N	Lung Cancer Human Plasma (NSCLC)	Hispanic 56/ Male	No/No	Negative	Nil	Nil	K2 EDTA
PL1022 19P	Lung Cancer Human Plasma (NSCLC)	Hispanic 68/ Female	No/No	Negative	Nil	Nil	K2 EDTA
PL1022 19Q	Lung Cancer Human Plasma (NSCLC)	Hispanic 57/ Female	No/No	Negative	Nil	Nil	K2 EDTA
PL1022 19R	Lung Cancer Human Plasma (NSCLC)	Hispanic 63/ Male	No/No	Negative	Nil	Nil	K2 EDTA
042115 D	Chronic Myelogenous Leukemia (CML) Human Serum	Caucasian 60/ Male	No/No	Negative	Nil	Nil / 23.5	Nil
042115 D	Chronic Myelogenous Leukemia (CML) Human Plasma	Caucasian 60/ Male	No/No	Negative	Nil	Nil / 23.5	Sodium Heparin

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 Camrelizumab Recovery

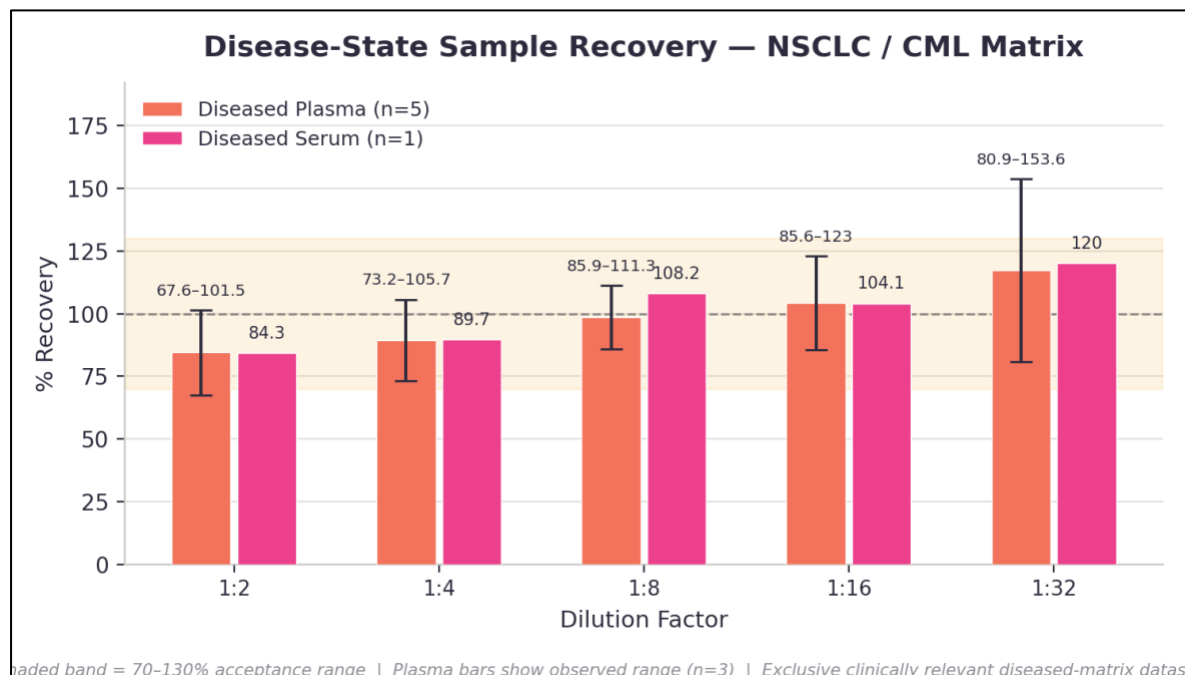
Sample Matrix	1:2 Dilution (%)	1:4 Dilution (%)	1:8 Dilution (%)	1:16 Dilution (%)	1:32 Dilution (%)
Diseased Serum (n=1)	84.3	89.7	108.2	104.1	120
Diseased Plasma (n=5)	67.6 – 101.5	73.2 – 105.7	85.9 – 111.3	85.6 – 123	80.9 - 153.6

Observations:

- Recovery of Camrelizumab in diseased serum remained within or near the acceptance range across all tested dilution levels, demonstrating robust assay performance and good tolerance to the diseased serum matrix.
- Diseased plasma samples showed acceptable recovery across the majority of dilution levels tested, confirming the assay's suitability for quantification in plasma matrices.
- Most plasma recovery values remained within the acceptance criterion, with only isolated high recoveries observed at higher dilution factors (1:16 and 1:32), suggesting that assay performance is strongest at lower to moderate dilutions.
- The assay demonstrated reliable analyte recovery across diverse diseased sample matrices, highlighting its ability to accurately detect Camrelizumab in clinically relevant samples.

Based on the observed results, the Camrelizumab ELISA Kit exhibits satisfactory recovery characteristics in both diseased serum and plasma samples, supporting its application for quantitative analysis within the recommended dilution range

Figure 5. Disease-State Sample Recovery — Diseased Plasma vs. Diseased Serum



6. 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.

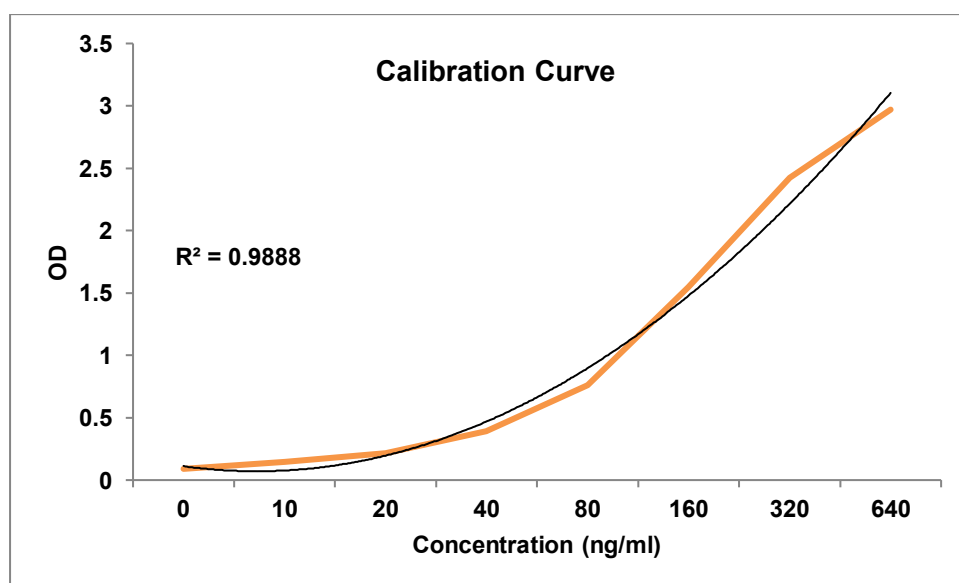
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at $1000 \times g$ within 30 minutes of collection. Assay immediately or store plasma samples at temperature $<-20^{\circ}\text{C}$. Avoid repeated freeze/thaw cycles.

B.) Storage conditions:

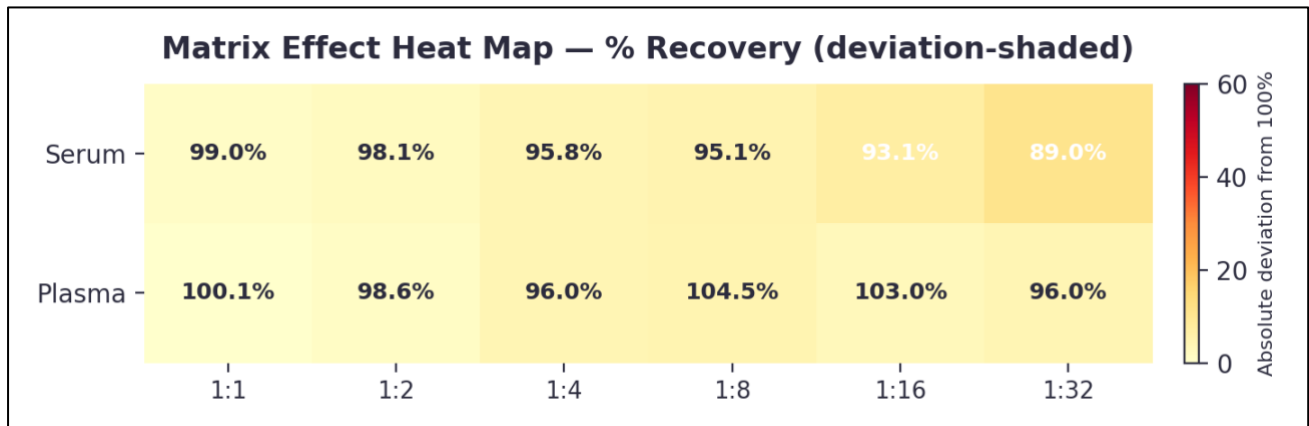
Store main kit components at $2-8^{\circ}\text{C}$.

Store recombinant lyophilized standard at $2-8^{\circ}\text{C}$. Upon reconstitution aliquot standards into polypropylene vials and store at -20°C as per assay requirements. Do not freeze thaw for more than two times. Before using, bring all components to room temperature ($18-25^{\circ}\text{C}$). Upon assay completion return all components to appropriate storage conditions.

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%

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