ELISA VALIDATION GUIDE

KRIBIOLISA® DARATUMUMAB (DARZALEX ™) ELISA

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

Background

1. Introduction to Daratumumab (DARZALEX™)

Daratumumab is a fully human monoclonal IgG1κ antibody that specifically targets CD38, a multifunctional ectoenzyme highly expressed on malignant plasma cells. By binding with high affinity to CD38, Daratumumab triggers multiple immune-mediated mechanisms of tumour cell elimination—including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and direct apoptosis via Fc-mediated crosslinking. These actions collectively result in potent depletion of pathogenic plasma cells and modulation of the tumour microenvironment. Clinically, Daratumumab is an essential therapy for multiple myeloma (MM) and light-chain (AL) amyloidosis, used across treatment lines as monotherapy or in combination with immunomodulators, proteasome inhibitors, and corticosteroids. The antibody was rationally engineered to exploit CD38's high expression on plasma cells while minimally affecting normal tissues. Its ability to engage both innate and adaptive immune mechanisms provides durable plasma-cell clearance and enhances antitumor immunity. Because CD38 is a common surface marker in plasma-cell disorders rather than a disease-specific antigen, Daratumumab is effective across a wide spectrum of clonal plasma-cell malignancies and continues to be evaluated for additional CD38-expressing hematologic conditions.

Daratumumab plays a pivotal role in immuno-oncology and haematology research, enabling detailed studies of plasma-cell biology, immune effector mechanisms, and monoclonal antibody-mediated cytotoxicity. Its monitoring involves assays such as flow cytometry for CD38 expression, serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE), minimal residual disease (MRD) testing, and pharmacokinetic ELISAs. In research environments, it serves as a model drug for exploring therapeutic antibody mechanisms, immune evasion, and micro environmental modulation in myeloma. Commercially available worldwide, Daratumumab is one of the most transformative biologics in plasma-cell dyscrasia management. It continues to be assessed in next-generation therapeutic strategies, subcutaneous formulations, combination regimens, and early-line treatment algorithms aimed at improving survival outcomes in myeloma and related disorders.

Daratumumab received its first regulatory approval from the U.S. Food and Drug Administration (FDA) in November 2015 under the brand name DARZALEX, for the treatment of relapsed or refractory multiple myeloma (RRMM) in patients who had received at least three prior lines of therapy. Its indications expanded rapidly based on strong clinical evidence demonstrating substantial progression-free and overall survival benefits. Over subsequent years, Daratumumab was approved for newly diagnosed MM (both transplant-eligible and ineligible populations), for relapsed/refractory disease in various combinations, and for treatment of AL amyloidosis. These approvals firmly established Daratumumab as a cornerstone therapy across multiple stages of plasma-cell disorders.

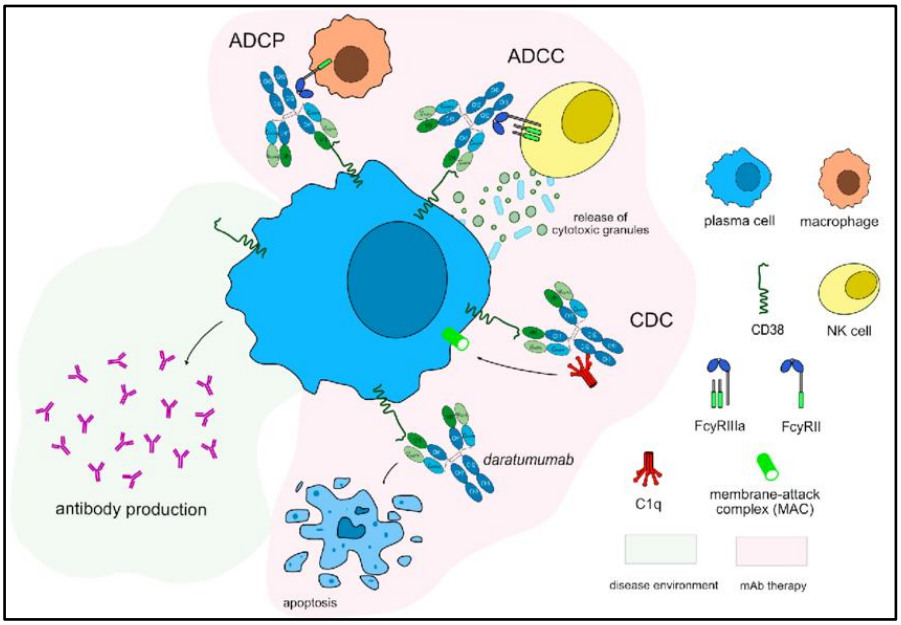


Figure 1: Immune response of Daratumumab.

2. Clinical Relevance of Daratumumab-Based Monitoring

Therapeutic drug monitoring (TDM) of Daratumumab is increasingly recognized as an important component of optimizing treatment outcomes, particularly in patients with variable tumor burden, altered immune effector function, or disease biology that affects antibody clearance and target saturation. Quantifying circulating Daratumumab levels enables accurate assessment of its pharmacokinetic (PK) behavior, depth of CD38 target engagement, and durability of plasma-cell depletion—key determinants of treatment response and long-term disease control in multiple myeloma (MM) and related plasma-cell disorders.

Monitoring drug exposure through TDM also helps correlate Daratumumab concentrations with clinically meaningful endpoints such as reduction in M-protein levels, improvement in hematologic parameters, normalization of free light chains, deeper MRD negativity rates, and sustained suppression of malignant plasma cells. These PK–PD relationships are particularly valuable in identifying patients with high tumor burden or accelerated drug consumption who may require dosing intensification, as well as distinguishing suboptimal responders despite standard dosing.

In addition, Daratumumab TDM is relevant for managing analytical interferences, such as false-positive immunofixation electrophoresis (IFE) or serum protein electrophoresis (SPEP) results, where the therapeutic antibody can mimic endogenous M-protein. Monitoring helps clinicians interpret laboratory findings accurately and avoid misclassification of response categories.

Ultimately, monitoring Daratumumab levels supports a personalized therapeutic approach by aligning CD38 blockade with individual disease kinetics, tumour load, and immunologic characteristics. This reduces the risk of inadequate target saturation, delayed therapeutic response, or unnecessary exposure once deep remission is achieved. Such individualized

dosing strategies improve treatment precision, enhance long-term survival outcomes, and support optimal management of plasma-cell malignancies.

Scope of Validation

This document presents a discussion of the characteristics of our KRIBIOLISA® Daratumumab ELISA KIT (CATALOG NO. KBI1028) 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 Daratumumab.
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).
- Matrix Effect (serum, plasma).
- Accelerated stability Study.
- Sample Handling and Storage Conditions.
- References Daratumumab Values and Recommended ELISA Range.

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® Daratumumab ELISA KIT, designed specifically to quantify free, active Daratumumab molecules with high sensitivity and specificity for accurate therapeutic drug monitoring. This assay enables precise measurement of circulating Daratumumab levels to support clinical research, pharmacokinetic (PK) profiling, dose individualization, and assessment of treatment efficacy in plasma-cell disorders.

The ELISA facilitates reliable detection of Subtherapeutic drug exposure, insufficient CD38 target engagement, or accelerated drug clearance—critical indicators for optimizing dosing strategies in multiple myeloma (MM) and AL amyloidosis. By providing quantitative insight into Daratumumab concentrations, the assay strengthens clinical decision-making related to treatment response, depth of plasma-cell depletion, and risk of disease progression or relapse. This kit is intended for use in research and clinical investigation settings that require robust, reproducible quantification of Daratumumab to support therapeutic drug monitoring, PK/PD correlation studies, and evaluation of antibody-mediated tumour cell elimination.

Principle of the Assay

This ELISA is based on a sandwich immunoassay format. Anti-Daratumumab capture antibodies are immobilized onto the wells of a 96-well microplate. Daratumumab present in the standards and test samples specifically binds to the coated antibodies during the incubation step. After washing to remove unbound materials, an HRP conjugated-Anti Daratumumab detection antibody is added, which binds to the captured Daratumumab, forming a stable antibody–antigen–antibody complex. Following further washing to eliminate excess conjugate, TMB substrate is introduced, enabling HRP to catalyze a colorimetric reaction. The reaction is stopped by adding stop solution, resulting in a yellow color. The measured optical density (OD) at 450 nm is directly proportional to the concentration of Daratumumab in the samples or standards.

Experimental Design

- A Sandwich ELISA was performed using Anti-Daratumumab monoclonal antibody as the capture antibody.
- Standards were prepared using purified Daratumumab reference material.
- Assay Concentration Range: 0 - 2560 ng/ml.
- Signal (% absorbance) plotted versus concentration.
- The optimized antibody-coating and detection strategy employed in the KRIBIOLISA® Daratumumab ELISA ensures efficient capture of free, pharmacologically active Daratumumab molecules while minimizing background signals from endogenous IgG, CD38-expressing fragments, or other serum components. This highly specific design provides excellent assay sensitivity and selectivity, making it well-suited for research applications as well as clinical monitoring of therapeutic drug levels, target engagement, and treatment response in patients receiving Daratumumab therapy.

The KRIBIOLISA® Daratumumab ELISA utilizes a quantitative sandwich immunoassay format based on the selective interaction between drug-specific monoclonal antibodies that recognize Daratumumab with high affinity. Anti- Daratumumab antibodies are pre-coated onto microwells to act as capture molecules. Patient samples and Daratumumab

standards are added, permitting the drug to bind to the immobilized capture antibodies. An HRP-conjugated anti- Daratumumab detection antibody is then applied to form a stable antibody–antigen–antibody complex. After washing to remove unbound material, TMB substrate is added, generating a colorimetric signal proportional to the amount of Daratumumab present in the sample. The reaction is stopped using stop solution, and absorbance is measured at 450 nm, providing a reliable quantitative determination of circulating Daratumumab levels.

Validation Parameters and Acceptance Criteria

1. Daratumumab Values and Recommended ELISA Range

This table summarizes levels across different therapies and suggested corresponding ELISA working ranges.

Application	Expected Daratumumab Range (ug/ml)	Recommended ELISA Range (ug/ml)
Post low-dose / induction phase (initial tumor burden reduction or priming doses)	5–20	0–50
Standard therapeutic maintenance dosing (routine clinical treatment cycles)	20–100	0–150
High-tumor-load states or intensified regimens (relapsed/refractory multiple myeloma; combination therapies)	80–200	0–250
Pharmacokinetic monitoring / target engagement-response evaluation	150–300	0–400

Note: Assay sensitivity <2 ng/mL is recommended for baseline or trough-level detection of Daratumumab, while an upper quantification limit of ≥200–300 ng/mL is advised for monitoring therapeutic exposure, evaluating target saturation (CD38 occupancy), and assessing potential loss of response or pharmacokinetic variability during standard or intensified dosing regimens.

The KRIBIOLISA® Daratumumab ELISA kit is developed using an assay range of 0 - 2560 ng/ml with the dilutional linearity accuracy to measure responses as per the application table above on patient C_{max} values. The kit has also been validated upto 3200 fold dilution and the values are within the acceptable range.

2. Specificity and Selectivity

2.1 Specificity

The capture and detection antibodies used in the Daratumumab ELISA are monoclonal antibodies that specifically recognize the intact human IgG1κ anti-CD38 therapeutic antibody without cross-reacting with endogenous human immunoglobulins or serum CD38-positive fragments. These assay antibodies are engineered to target unique idiotype or Fab-region epitopes of Daratumumab, ensuring high-affinity binding to the drug molecule in its native, functional conformation.

The optimized specificity profile enables selective detection of Daratumumab in complex biological matrices—including serum, plasma, or cell-culture supernatants—while demonstrating minimal interference from endogenous IgG, soluble CD38, CD38-expressing micro vesicles, or structurally unrelated monoclonal antibodies used in combination regimens. This high level of molecular discrimination ensures accurate quantification of circulating Daratumumab even in patients with high tumor burden, immune activation, or CD38-rich microenvironments, provided the drug's idiotype integrity is preserved.

2.2 Selectivity

The ELISA demonstrates minimal to no cross-reactivity with endogenous human IgG subclasses, recombinant antibodies, or unrelated therapeutic monoclonal antibodies. It effectively excludes molecules that do not share the unique idiotype or antigenic determinants of Daratumumab, including soluble CD38, CD38-expressing microvesicles, CD38 ligands, or other surface antigens present in circulation.

The assay maintains high selectivity in complex biological matrices such as serum, plasma, or cell-culture supernatants, showing negligible interference from serum immunoglobulins, cytokines, inflammatory mediators, heterophilic antibodies, Fc-binding proteins, or CD38-rich matrix components. This stringent selectivity ensures reliable and accurate quantification of Daratumumab without false-positive signals arising from structurally unrelated monoclonal antibodies used in combination therapies or endogenous CD38-associated proteins.

2.3LOD, LOQ and IC_{50}

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® Daratumumab ELISA = 8.76 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).

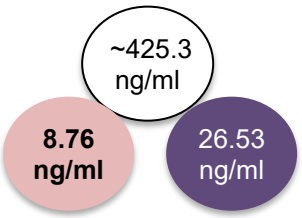
LOQ for KRIBIOLISA® Daratumumab ELISA – 26.53 ng/ml

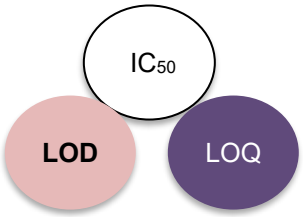
IC_{50} in ELISA (Half Maximal Inhibitory Concentration)
IC_{50} = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

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.

IC_{50} for KRIBIOLISA® Daratumumab ELISA = ~425.3 ng/ml
Summary:

Parameter	Value (ng/ml)
LOD	8.76 ng/ml
LOQ	26.53 ng/ml
IC_{50}	425.3 ng/ml



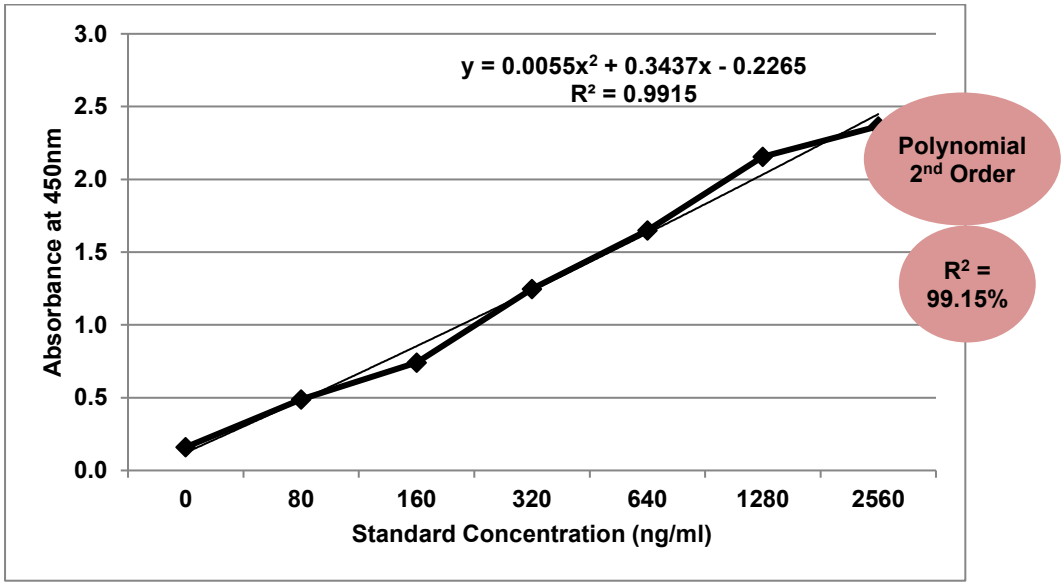


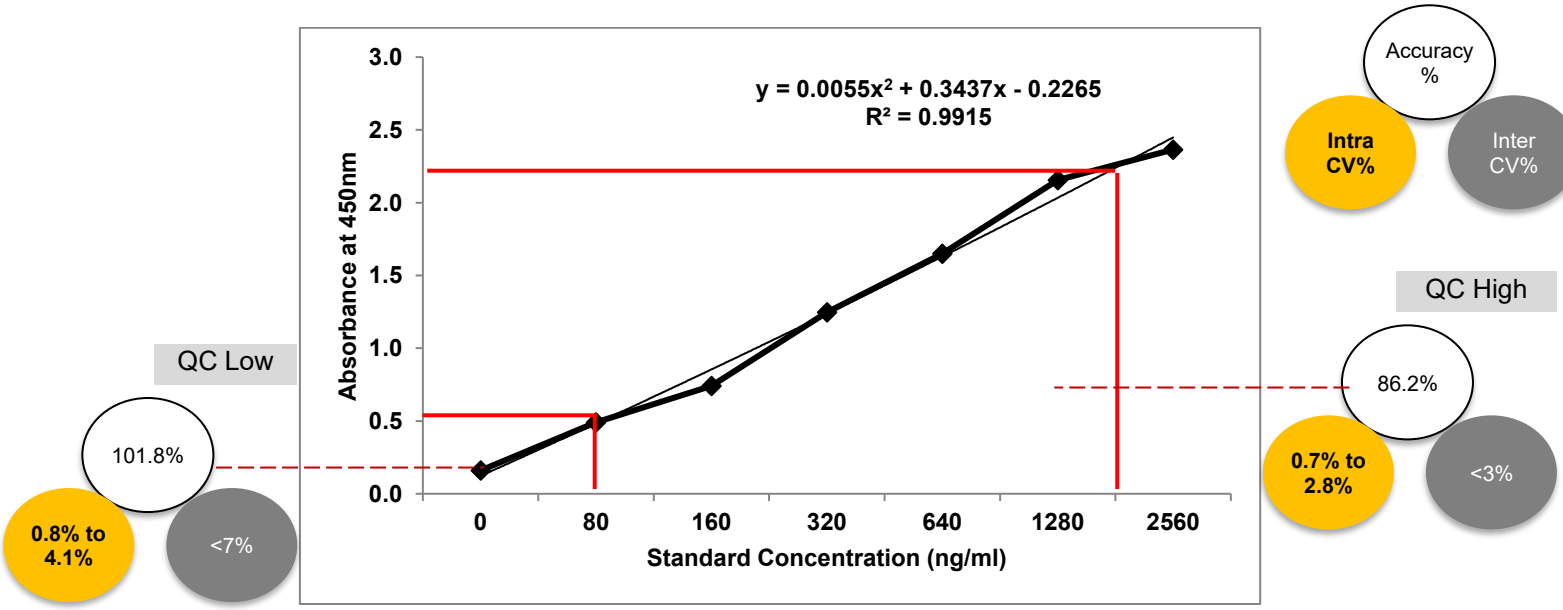
Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
0	0.16	--	--
80	0.489	83.2	104
160	0.742	151.8	94.9
320	1.247	339.3	106
640	1.649	594.7	92.9

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
1280	2.155	1396.5	109.1
2560	2.365	2429.6	94.9
Positive Control (2500 ng/ml)	2.359	2381.8	95.3
Low QC Control (80 ng/ml)	0.482	81.4	101.8
High QC Control (2000 ng/ml)	2.246	1724.6	86.2





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

Precision was assessed by analyzing three standard concentrations (80 ng/ml, 640 ng/ml, and 2560 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 QC samples.
- Inter-assay %CV should be ≤15% for QC 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
80	0.8% to 4.1%	<7%
640	0.6% to 5.9%	<6%
2560	0.7% to 2.8%	<3%

Observations:

- Intra-assay precision was consistently less than 7% across all levels tested.
- Inter-assay precision was consistently less than 7%.
- All precision values met the acceptance criteria for ELISA validation.

Conclusion:

The KRIBIOLISA® Daratumumab 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.

5. Diluents Effect Study

Evaluation of PBS-based buffer vs Proprietary buffer revealed slight recovery differences. PBS (pH 7.4) diluent offered consistent and reliable performance across tested concentrations.

6. Parallelism

Serial dilutions of a high-concentration sample were prepared at dilutions of 1:200, 1:400, 1:800, 1:1600 and 1:3200 for both human serum and human plasma. Each dilution was assayed using the KRIBIOLISA® Daratumumab ELISA and compared to the standard curve.

Acceptance Criteria:

- The back-calculated concentration (interpolated) should fall within ±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
1:200	1280	2.147	1372.9	107.3	93.2
1:400	640	1.655	599.8	93.7	106.7
1:800	320	1.27	350.4	109.5	91.3
1:1600	160	0.744	152.4	95.2	105
1:3200	80	0.432	69	86.2	116

B) Human Plasma:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:200	1280	2.263	1218.4	95.2	105.1
1:400	640	1.745	600.2	93.8	106.6
1:800	320	1.387	360.4	112.6	88.8

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1600	160	0.851	141.9	88.7	112.7
1:3200	80	0.566	68.8	86.1	116.2

Results:

i. Parallelism is generally maintained across the 1:200 to 1:3200 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® Daratumumab 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 Daratumumab ELISA without significant loss of accuracy.

7. 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 (0–2560 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 + 1:100 Human Serum)	% Standard Deviation	% CV
0	0.146	0.16	1.0	6.5
80	0.99	0.489	35.37	47.8
160	1.571	0.742	58.62	50.7

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:100 Human Serum)	% Standard Deviation	% CV
320	2.052	1.247	56.87	34.5
640	2.494	1.649	59.77	28.8
1280	2.668	2.155	36.32	15.1
2560	3.119	2.365	53.33	19.5

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:100 Human Plasma)	% Standard Deviation	% CV
0	0.146	0.124	2.52	17.7
80	0.99	0.598	7.69	14.1
160	1.571	0.852	7.78	9.8
320	2.052	1.412	11.65	8.8
640	2.494	1.789	9.89	5.8
1280	2.668	2.228	5.18	2.4
2560	3.119	2.789	30	11.6

Results:

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

Conclusion:
The KRIBIOLISA® Daratumumab ELISA demonstrates excellent performance in the presence of human serum and plasma. The assay results confirm the absence of significant matrix interference, supporting its reliability for analysing biological samples.

8. Accelerated Stability Study

Accelerated stability studies in ELISA are performed to predict the shelf life and long-term stability of an ELISA kit or its individual components by exposing them to elevated stress conditions (typically higher temperatures) for a defined period.

The following table demonstrates the relation of temperature with time point and number of days:

Accelerated Study Day (37 degrees)	Real-Time Equivalent Age (2-8 degree)	Interpretation
Day 0	Present day (0 months)	Initial / release testing
Day 1	26 days (Approx. 1 month)	Early stability checkpoint
Day 4	104 days (Approx. 3.5 months	Short-term stability trend
Day 7	182 days (Approx. 6 months)	Mid-term shelf-life prediction
Day 14	364 days (Approx. 1 year)	One-year shelf-life equivalence

Accelerated Stability Study data:

Standard Concentration (ng/ml)	Absorbance (Day 0)	Absorbance (Day 1)	Absorbance (Day 4)	Absorbance (Day 7)	Absorbance (Day 14)	%CV
0	0.235	0.222	0.251	0.234	0.225	5.0
80	0.670	0.666	0.653	0.567	0.481	13.6
160	0.896	0.924	1.024	0.878	0.712	12.7
320	1.353	1.415	1.551	1.401	1.026	14.5
640	1.969	1.988	2.168	1.991	1.505	12.9
1280	2.355	2.324	2.224	2.335	2.002	6.5
2560	2.770	2.690	2.595	2.515	2.284	7.3

Results:

I. %CV is less than 15% across all days.
II. Based on the accelerated stability study results, the Daratumumab ELISA kit demonstrates satisfactory stability and robustness, supporting its viability with an extended shelf life and an assigned expiry of 1 year under recommended storage conditions.

9. Sample Handling and Storage Conditions

A) Specimen Collection and Handling:

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be

run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

For Cell Culture Supernatant **–** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80ºC. Avoid repeated freeze-thaw cycles.

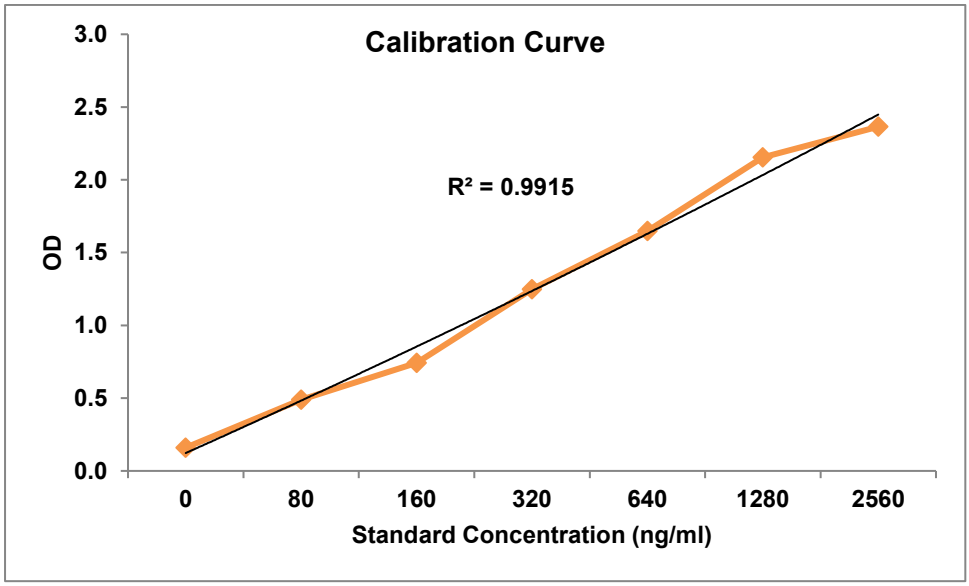
B) Handling / Storage:

- It is advisable to aliquot and store the Anti- Daratumumab:HRP Conjugate concentrated at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C. Immediately discard any excess working
- Anti-Daratumumab: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 (18-25ºC). 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 sources.

C) Health Hazard Warnings:

- Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
- For Research Use Only

Graphs, Maps and Appendices:



Matrix Effect Heat Map

	1:200	1:400	1:800	1:1600	1:3200
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% (100 ± 25%	-
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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