ELISA VALIDATION GUIDE

KRIBIOLISA® ANTI-ASPART (Novolog™) ELISA

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

Background

1. Introduction to Anti-Insulin Asparte Antibodies

Anti-Insulin Asparte antibodies are immunoglobulins generated by the host immune system in response to exposure to Insulin Asparte, a rapid-acting insulin analog widely used for the management of diabetes mellitus. Insulin Asparte differs from native human insulin by a single amino acid substitution (proline replaced by Aspartic acid at position B28), a modification that reduces self-association and enables faster absorption following subcutaneous administration. Despite its close structural similarity to endogenous insulin, Insulin Asparte can induce an immune response in some individuals, leading to the formation of anti-drug antibodies (ADAs).

These antibodies may recognize linear or conformational epitopes within the insulin backbone or regions influenced by the B28 Aspartic acid substitution. The development of anti-Insulin Asparte antibodies has the potential to alter pharmacokinetics and pharmacodynamics by affecting insulin absorption, distribution, or clearance. In certain cases, antibody binding may attenuate insulin bioavailability, contribute to glycaemic variability, or result in delayed or unpredictable insulin action. While most antibodies detected during therapy are of low affinity and non-neutralizing, persistent or high-titer responses may have clinical relevance.

From a mechanistic standpoint, anti-Insulin Asparte antibodies provide valuable insight into insulin immunogenicity, structure–function relationships, and immune tolerance to recombinant insulin analogs. In both clinical and research settings, their detection and characterization are commonly performed using immunoassays such as bridging ELISA, competitive ELISA, electrochemiluminescence-based assays, and, where applicable, functional bioassays to assess neutralizing potential. These approaches enable sensitive measurement of ADA incidence, titer, binding specificity, and temporal dynamics during treatment.

Anti-Insulin Asparte antibodies are particularly significant in regulatory, pharmacovigilance, and biosimilars development contexts, where immunogenicity evaluation is a mandatory component of safety and comparability assessments. Monitoring ADA responses assists in interpreting inter-patient variability in glycaemic control, insulin dose requirements, and long-term treatment outcomes. Such data also support risk–benefit analysis and post-marketing surveillance of insulin analog therapies.

In preclinical and translational research, anti-Insulin Asparte antibodies contribute to the broader understanding of immune responses to insulin products and inform strategies to minimize immunogenicity through formulation optimization and molecular design. With the continued global reliance on insulin analogs for diabetes management and the development of next-generation insulin therapies, anti-Insulin Asparte antibodies remain a critical analytical and research tool for immunogenicity assessment, assay validation, and long-term safety evaluation.

2. Clinical Relevance of Anti-Insulin Asparte–Based Monitoring

Monitoring anti-Insulin Asparte antibodies is a critical component of immunogenicity assessment during Insulin Asparte therapy, particularly in patients exhibiting unexplained glycaemic variability, unexpected loss of prandial glucose control, or altered insulin pharmacokinetics. The development of anti-drug antibodies (ADAs) can influence insulin bioavailability and pharmacodynamic action by modifying absorption, increasing insulin clearance, or binding circulating insulin and delaying its release. Quantification of anti-Insulin Asparte antibodies therefore provides essential insight into treatment-related immune responses and their potential clinical consequences.

Assessment of ADA incidence, titer, and persistence enables correlation of immune reactivity with clinically relevant outcomes such as postprandial hyperglycaemia, increased glucose excursions, higher insulin dose requirements, or unpredictable insulin action profiles. In certain cases, high-titer or high-affinity antibodies may sequester Insulin Asparte, resulting in delayed onset of action or secondary hypoglycaemic episodes due to insulin dissociation. Anti-Insulin Asparte antibody monitoring also assists in distinguishing immunogenicity-related therapeutic variability from factors such as disease progression, injection technique issues, or non-adherence.

From a therapeutic management perspective, anti-Insulin Asparte antibody monitoring supports informed clinical decision-making, including insulin dose optimization, modification of dosing schedules, or transition to alternative insulin analogs with different molecular structures or immunogenicity characteristics. In patients receiving long-term insulin therapy, immunogenicity profiling helps identify sustained antibody responses that may compromise treatment predictability, metabolic stability, or overall glycaemic control.

Ultimately, anti-Insulin Asparte–based monitoring facilitates a personalized diabetes management approach by integrating immunogenicity data with patient-specific glycaemic patterns and clinical outcomes. This strategy enhances the reliability and long-term effectiveness of insulin therapy, supports regulatory pharmacovigilance and biosimilar comparability requirements, and contributes to optimized clinical management of diabetes while minimizing immune-mediated variability in therapeutic response.

Scope of Validation

This document presents a discussion of the characteristics of our KRIBIOLISA® Anti-Asparte (Novolog) ELISA KIT (CATALOG NO. KBI9003) 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 Anti-Asparte antibodies.
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 Anti-Asparte 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® Anti-Asparte (Novolog) ELISA KIT, designed specifically to detect and quantify anti-Insulin Asparte antibodies with high sensitivity and specificity. This assay enables accurate immunogenicity assessment during Insulin Asparte therapy by supporting the detection, characterization, and monitoring of anti-drug antibody (ADA) responses. It facilitates clinical research, pharmacovigilance, and treatment evaluation by enabling assessment of ADA incidence, titer, and persistence, as well as their potential impact on insulin exposure and glycaemic control.
The assay supports informed clinical decision-making by enabling identification of immune-mediated variability or loss of therapeutic response, guiding insulin dose optimization, regimen adjustment, or transition to alternative insulin analogs. It contributes to ensuring predictable and sustained glycaemic management in patients receiving Insulin Asparte for the treatment of diabetes mellitus, while supporting long-term safety monitoring and regulatory immunogenicity requirements.

Principle of the Assay

This ELISA is based on an indirect immunoassay format for the detection of anti-Insulin Aspart (NovoLog®) antibodies. Novo Rapid Penfill, Insulin Aspart I.P., 100 U/ml, is immobilized onto the wells of a 96-well microplate and serves as the capture antibody.

During incubation, Insulin Aspart Antibody present in standards, controls, or test samples specifically bind to the coated antibody. After a wash step to remove unbound substances, an Anti-Human IgG-HRP conjugated detection antibody is added, which binds to the captured Novo Rapid Penfill, Insulin Aspart I.P, forming a stable immune complex. Following additional washing to eliminate excess conjugate, TMB substrate is added, allowing horseradish peroxidase to catalyze a colorimetric reaction. The reaction is terminated by the addition of stop solution, producing a yellow colour. The optical density (OD) measured at 450 nm is directly proportional to the concentration of anti-Insulin Aspart antibodies present in the samples or standard

Experimental Design

- An indirect ELISA was performed using Novo Rapid Penfill, Insulin Aspart I.P., 100 U/ml as the capture antibody.
- Standards were prepared using purified Insulin Aspart Antibody reference material.
- Assay Concentration Range: 0 - 8000 ng/ml.
- Signal (% absorbance) plotted versus concentration.
- The optimized antibody-coating and detection strategy employed in the KRIBIOLISA® Anti-Aspart (Novolog) ELISA KIT enables highly specific capture of anti-Insulin Aspart antibodies while minimizing non-specific background arising from endogenous insulin, naturally occurring anti-insulin antibodies, or other serum components. This selective assay design provides excellent sensitivity and reproducibility, making it well suited for immunogenicity assessment in research applications and for monitoring antibody responses in patients receiving Insulin Aspart therapy.

The KRIBIOLISA® Anti-Asparte (Novolog) ELISA KIT utilizes an indirect immunoassay format based on the selective interaction between Insulin Aspart and anti-Insulin Aspart antibodies present in the sample. Novo Rapid Penfill, Insulin Aspart I.P., 100 U/ml capture antibodies are pre-coated onto microwells to act as capture molecules. Patient samples and Insulin Aspart Antibody standards are added, permitting the drug to bind to the immobilized capture antibodies. An Anti-Human IgG: HRP-conjugated detection antibody is then applied to form a stable immune complex. After washing to remove unbound material, TMB substrate is added, generating a colorimetric signal proportional to the amount of Insulin Aspart Antibody 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 Insulin Aspart Antibody levels.

Validation Parameters and Acceptance Criteria

1. Anti-Insulin Asparte antibodies Values and Recommended ELISA Range

This table summarizes Anti-Insulin Asparte antibodies levels across different therapies and suggested corresponding ELISA working ranges.

Application	Expected Anti-Insulin Asparte antibodies Range (ug/ml)	Recommended ELISA Range (ug/ml)
Baseline / pre-treatment screening (ADA-negative reference)	Not detectable – 0.1	0–1
Early treatment phase (low-titer or transient ADA response)	0.1–1	0–5
Sustained therapy with moderate ADA development (possible PK impact)	1–10	0–20
High-titer or persistent ADA response (potential insulin binding or delayed action)	10–100	0–200

Note: An assay with high analytical sensitivity is recommended for the reliable detection of low-titer anti-Insulin Aspart antibodies at baseline and during early treatment, including pre-existing or treatment-emergent immune responses. A sufficiently wide upper quantification range is advised to enable accurate measurement of high-titer anti-drug antibodies that may develop during prolonged or intensified Insulin Aspart therapy. These performance characteristics are important for comprehensive immunogenicity assessment, evaluation of potential effects on insulin pharmacokinetics and glycaemic control, and long-term monitoring of patient response.

The KRIBIOLISA® Anti-Insulin Aspart (NovoLog®) ELISA kit is developed using an assay range of 0 - 8000 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 8000 fold dilution and the values are within the acceptable range.

2. Specificity and Selectivity

2.1 Specificity

The capture and detection reagents used in the Anti-Insulin Asparte ELISA are highly specific antibodies and assay components designed to selectively recognize anti-Insulin Asparte antibodies without cross-reactivity to endogenous immunoglobulins or unrelated circulating antibodies. The assay is optimized to detect antibodies directed against Insulin Asparte–specific epitopes, including amino acid substitutions and conformational determinants unique to the rapid-acting insulin analog structure, ensuring high-affinity and selective binding of anti-drug antibodies present in patient samples.

The specificity profile enables accurate discrimination of anti-Insulin Asparte antibodies in complex biological matrices such as human serum or plasma while minimizing interference from endogenous human insulin, circulating free Insulin Asparte, insulin–albumin

interactions, or insulin metabolites. The assay demonstrates minimal cross-reactivity with other recombinant human insulins, long-acting or intermediate-acting insulin analogs, proinsulin, C-peptide, or unrelated peptide hormones commonly present in diabetic patient samples.
This high degree of molecular specificity ensures reliable detection and quantification of anti-Insulin Asparte antibodies across a wide range of antibody titers, including samples with elevated insulin exposure or metabolic background interference. Consequently, the assay supports robust immunogenicity assessment, pharmacovigilance, and clinical monitoring of immune responses during Insulin Asparte therapy, provided that antibody binding epitopes remain immunoreactive.

2.2 Selectivity

The Anti-Insulin Asparte ELISA demonstrates minimal to no cross-reactivity with endogenous human immunoglobulin subclasses, naturally occurring anti-insulin antibodies, or unrelated therapeutic monoclonal antibodies. The assay selectively detects antibodies directed against Insulin Asparte–specific antigenic determinants and effectively excludes antibodies that do not recognize the unique structural and conformational epitopes of the Insulin Asparte molecule, including epitopes shared with endogenous human insulin or other insulin analogs.
The assay maintains high selectivity in complex biological matrices such as human serum, plasma, or cell-culture supernatants, with negligible interference from endogenous insulin, circulating free Insulin Asparte, insulin–albumin interactions, insulin metabolites, proinsulin, C-peptide, or other peptide hormones and metabolic regulators commonly present in diabetic patient samples. In addition, minimal cross-reactivity is observed with rapid-acting, long-acting, or intermediate-acting insulin analogs, ensuring discrimination of anti-Insulin Asparte antibodies from immune responses directed against other insulin therapies.
This stringent selectivity profile ensures reliable and accurate detection of anti-Insulin Asparte antibodies without false-positive signals arising from biologically active matrix components or therapeutically related agents, thereby supporting robust immunogenicity assessment and clinical monitoring during Insulin Asparte therapy.

2.3 LOD, 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® Anti-Insulin Aspart (NovoLog®) ELISA = 115.20 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).

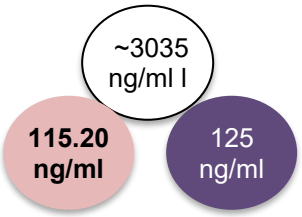
LOQ for KRIBIOLISA® Anti-Insulin Aspart (NovoLog®) ELISA – 125 ng/ml

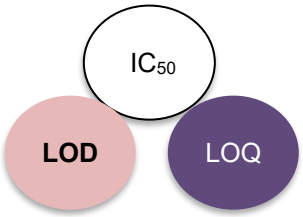
IC_{50} in ELISA (Half Maximal Inhibitory Concentration)
IC_{50} = The concentration of an inhibitor (drug, antibody, compound) required to reduce the signal (e.g., binding, enzymatic activity) by 50% compared to the maximum signal in the assay.

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® Anti-Insulin Aspart (NovoLog®) ELISA = ~3035 ng/ml
Summary:

Parameter	Value (ng/ml)
LOD	115.20 ng/ml
LOQ	125ng/ml
IC_{50}	3035ng/ml



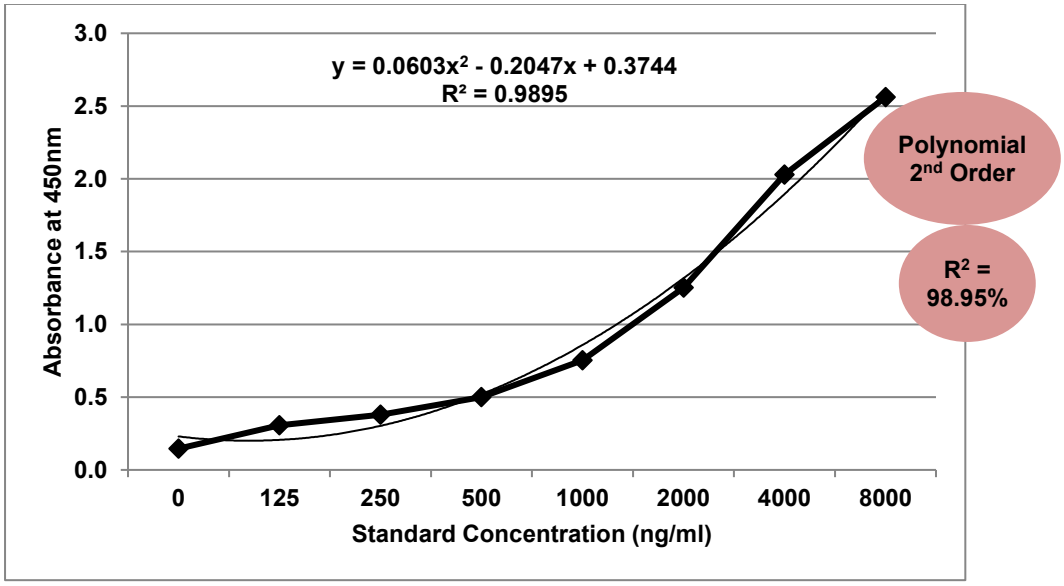


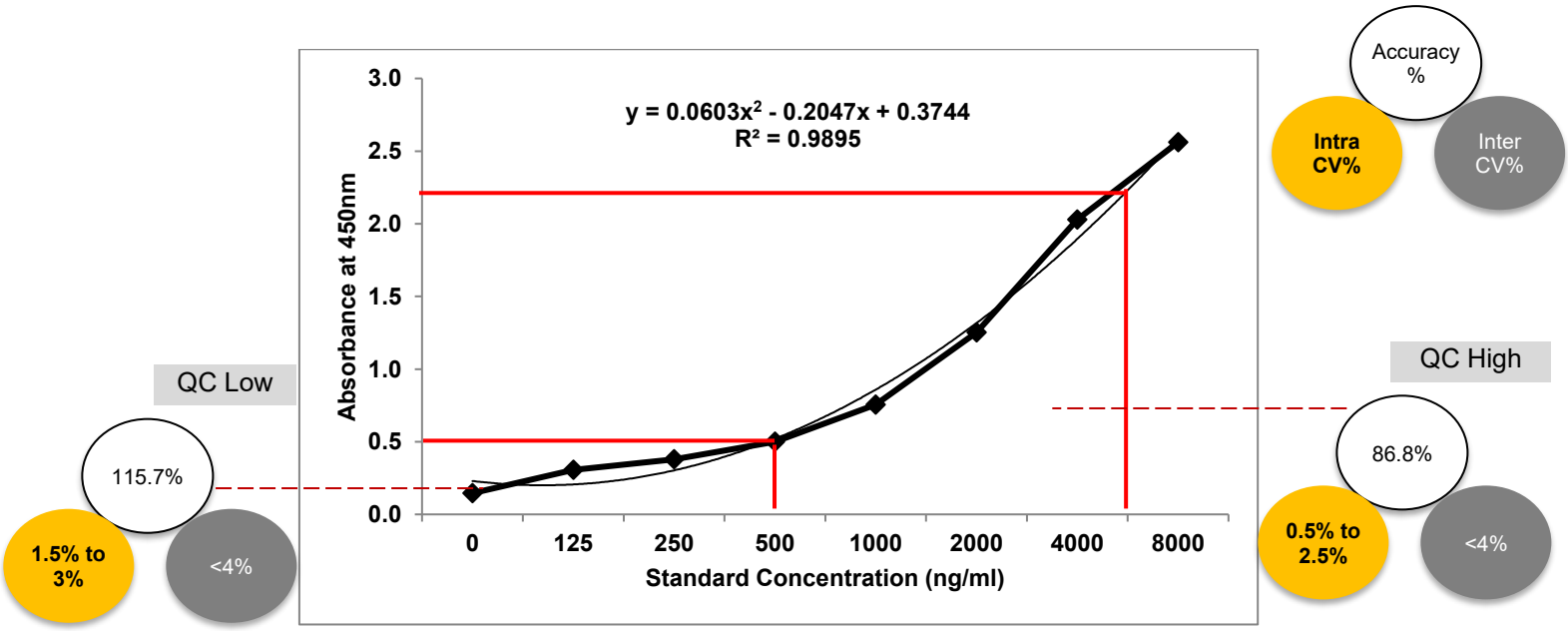
Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
0	0.146	--	--
125	0.307	125.4	100.3
250	0.379	307	122.8
500	0.499	545.5	109.1
1000	0.754	995.7	99.6
2000	1.252	1920.9	96
4000	2.028	4153.2	103.8

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
8000	2.561	7839.2	98
Positive Control (7000 ng/ml)	2.539	7128.9	101.8
Low QC Control (500 ng/ml)	0.518	578.7	115.7
High QC Control (6000 ng/ml)	2.258	5208.7	86.8





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

Precision was assessed by analyzing three standard concentrations (250 ng/ml, 2000 ng/ml, and 8000 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
250	0.7% to 1.1%	<3%
2000	0.8% to 2.2%	<3%
8000	0.9% to 1.5%	<2%

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® Anti-Insulin Aspart (NovoLog®) 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 1:2000, 1:4000, 1:8000, 1:16000, 1:32000 and 1:64000 for both human serum and human plasma. Each dilution was assayed using the KRIBIOLISA Anti-Insulin Aspart (NovoLog®) 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
1:2000	4000	2.033	4690.8	117.3	117.3
1:4000	2000	1.269	2091.7	104.6	104.6
1:8000	1000	0.762	1074.2	107.4	107.4
1:16000	500	0.282	192	38.4	38.4
1:32000	250	0.197	---	0	0
1:64000	125	0.146	---	0	0

B) Human Plasma:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:2000	4000	2.060	4390.6	109.8	120.9
1:4000	2000	1.261	1918.9	95.9	103.7

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:8000	1000	0.753	952.8	95.3	105.8
1:16000	500	0.301	192.6	38.5	46.9
1:32000	250	0.208	---	0.0	0.0
1:64000	125	0.157	---	0.0	0.0

Results:

i. Parallelism is generally maintained across the 1:2000 to 1:64000 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® Anti-Insulin Aspart (NovoLog®) 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 Anti-Insulin Aspart (NovoLog®) 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 (0–8000 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	0.131	0.146	0.010	7.4

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:1000 Human Serum)	% Standard Deviation	% CV
125	0.3	0.307	0.005	1.6
250	0.342	0.379	0.026	7.3
500	0.5	0.499	0.001	0.1
1000	0.781	0.754	0.019	2.5
2000	1.213	1.252	0.027	2.2
4000	1.938	2.028	0.064	3.2
8000	2.615	2.561	0.038	1.5

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:1000 Human Plasma)	% Standard Deviation	% CV
0	0.131	0.181	0.024	15
62.5	0.3	0.254	0.037	13.4
125	0.342	0.35	0.021	5.6
250	0.5	0.467	0.023	4.7
500	0.781	0.684	0.049	6.9
1000	1.213	1.218	0.024	1.9
2000	1.938	1.912	0.082	4.2
4000	2.615	2.441	0.085	3.4

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® Anti-Insulin Aspart (NovoLog®) 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 analyzing 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	0.292	0.259	0.260	0.223	0.252	9.5
125	0.410	0.401	0.380	0.358	0.322	9.4
250	0.555	0.548	0.532	0.464	0.429	11.1
500	0.707	0.745	0.665	0.652	0.553	10.9
1000	1.131	1.234	1.084	1.123	0.834	13.8
2000	1.762	1.754	1.731	1.686	1.379	9.7
4000	2.391	2.295	2.498	2.398	2.116	6.2
8000	2.655	2.687	2.790	2.625	2.712	2.3

Results:

I. %CV is less than 15% across all days.
II. Based on the accelerated stability study results, the Anti Aspart 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. Cross Reactivity:

To assess the cross-reactivity and specificity of the KRIBIOLISA Anti-Insulin Aspart (NovoLog®) ELISA, a comparative evaluation was conducted using Human Insulin alongside Anti-Insulin Aspart.

The following table demonstrates Anti-Insulin Aspart standard readings:

Standard concentration (ng/ml)	Abs 1	Abs 2	Mean Absorbance	Interpolated Concentration	% Recovery	Signal Difference
0	0.136	0.156	0.146	--	--	--
125	0.306	0.308	0.307	125.4	100.3	0.17
250	0.381	0.377	0.379	307	122.8	0.075
500	0.505	0.493	0.499	545.5	109.1	0.124
1000	0.777	0.731	0.754	995.7	99.6	0.272
2000	1.246	1.257	1.252	1920.9	96	0.469
4000	2.016	2.04	2.028	4153.2	103.8	0.77
8000	2.621	2.501	2.561	7839.2	98	0.605

The following table demonstrates -Human Insulin antibody standard readings:

Standard	Human Insulin antibody		
Standard Concentration (ng/ml)	**Mean Absorbance**	**Interpolated Concentration**	**% Recovery**
0	0.213	Below Detection range	--
125	0.210	Below Detection range	0.0
250	0.229	3.3	1.3
500	0.201	Below Detection range	0.0
1000	0.219	Below Detection range	0.0
2000	0.242	27.7	1.4
4000	0.270	76.9	1.9
8000	0.578	611.1	7.6

Comparison between Anti Asparte and Human Insulin antibody Interpolated concentration:

Standard	Anti Asparte	Human Insulin antibody	
Standard Concentration (ng/ml)	**Interpolated Concentration**	**Interpolated Concentration**	**% Cross Reactivity**
0		Below Detection range	--
125	125.4	Below Detection range	0
250	307.0	3.3	1.1

Standard Concentration (ng/ml)	Interpolated Concentration	Interpolated Concentration	% Cross Reactivity
500	545.5	Below Detection range	0
1000	995.7	Below Detection range	0
2000	1920.9	27.7	1.4
4000	4153.2	76.9	1.9
8000	7839.2	611.1	7.8

Results:

The table demonstrates that Anti-Insulin Aspart (NovoLog®) ELISA has no cross reactivity with Human Insulin.

10. 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.

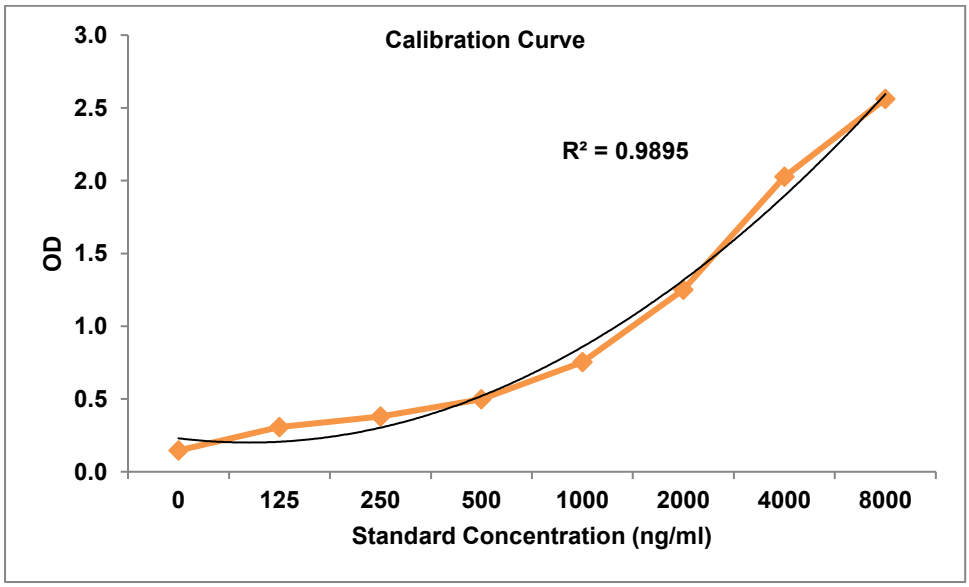
B) Handling / Storage:

- It is advisable to aliquot and store the Anti- Human IgG 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- Human IgG: 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

	1:2000	1:4000	1:8000	1:16000	1:32000	1:64000
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%

References

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