ELISA VALIDATION GUIDE

KRIBIOLISA® Double-Stranded RNA (dsRNA) ELISA (J2 based)

KRISHGEN BioSystems

OUR REAGENTS, YOUR RESEARCH

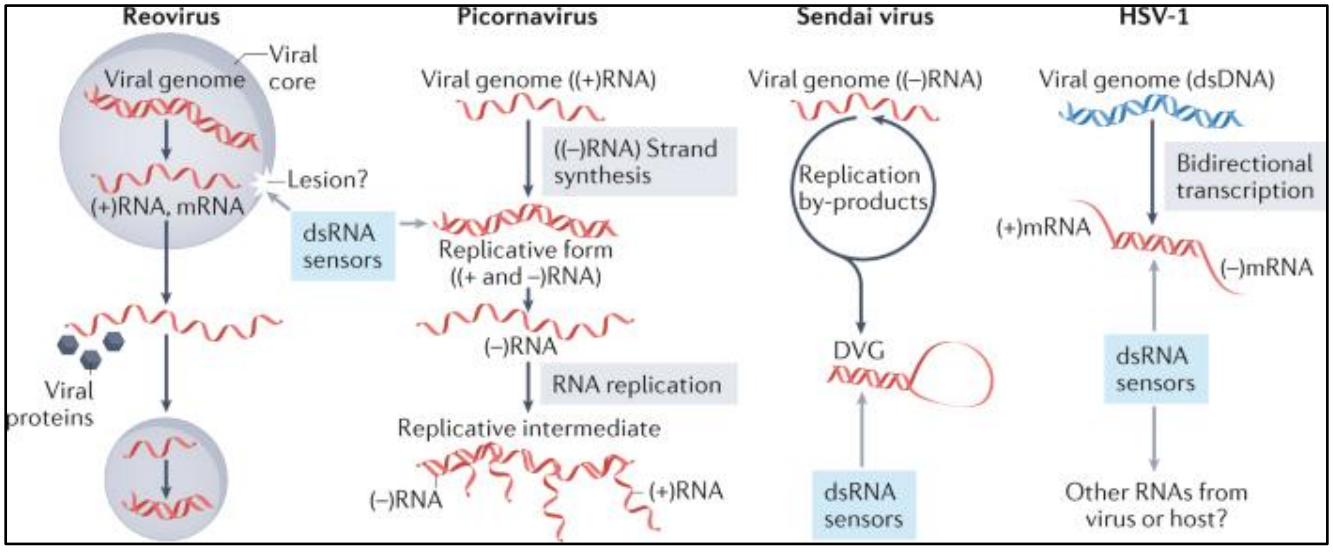
Background

1. **Introduction to Double-Stranded RNA (dsRNA) J2 based**

Double-stranded RNA (dsRNA) J2 antibody is a type of monoclonal antibody that specifically recognizes to double stranded RNA molecules and binds to the same which are associated with viral replication or aberrant structural RNA in the host cells. It is primarily used in research and diagnostic applications, significantly for viral infection detections, assessment of innate immune responses and investigation of RNA interference (RNAi) pathways. The J2 antibody was designed in such a way so that it can bind with high affinity and specificity to dsRNA structures of at least 40 base pairs or more than that. The J2 antibody recognizes dsRNA in a sequence-independent manner unlike sequence-specific nucleic acid probes, which makes it a powerful and strong tool for identification of diverse viral RNA species and synthetic dsRNA analogs like poly(I:C).

J2 antibody plays a significant role in biomedical research, especially in the diverse fields of virology, innate immunity, and RNA-based therapeutics. Its binding to dsRNA activates a cascade of downstream detection pathways such as immunofluorescence, ELISA, and immunoblotting. In innate immunity studies, J2 helps visualize the presence of viral dsRNA intermediates, which are considered as potential activators of pattern recognition receptors (PRRs) like RIG-I, MDA5, and TLR3.

The J2 monoclonal antibody is commercially available in the market and widely used in academic and industrial laboratories. It is often used alongside other antibodies and markers in immunocytochemistry, immunoprecipitation, and microscopy-based detection systems to monitor antiviral responses, RNA editing, and synthetic RNA delivery.



2. **Clinical Relevance of Double-Stranded RNA (dsRNA) J2 based Monitoring**

Therapeutic drug monitoring or TDM of dsRNA J2 based therapies are important for rationally optimizing the dosage regimens especially in patients who are suffering from variable immune responses or some underlying diseases conditions where their RNA

metabolism is affected. Adequate monitoring of dsRNA J2 levels helps in assessing comprehensive pharmacokinetic (PK) profiling, immunostimulatory potency and therapeutic efficacy evaluation which helps in decreasing the risk of systemic inflammation. In addition to this, TDM helps in correlating dsRNA J2 to the required clinical outcomes like enhanced interferon response or tumor suppression. This helps in facilitating personalized treatment strategies in response to cancer or antiviral immunotherapy.

Scope of Validation

This document presents a discussion of the characteristics of our KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA (J2 based) KIT (CATALOG NO. KBBA56) 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 ds RNA..
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).
- Sample Handling and Storage Conditions.
- References (Double-Stranded RNA (dsRNA) J2 based 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 assess the specificity, assay performance, and clinical relevance of the KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA developed using synthetic double-stranded RNA (dsRNA) analogs which mimics the structural features of cellular and viral dsRNA that are identified by J2 monoclonal antibody.

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.

Experimental Design

- A Sandwich ELISA was performed using J2 monoclonal antibody as capture antibody.
- Standards prepared for synthetic double-stranded RNA (dsRNA) analogs.
- Assay Concentration Range: 0 - 100 ng/ml.
- Signal (% absorbance) plotted versus concentration.
- The specific dsRNA immobilization strategy used in the KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA enhances and improves the binding efficiency of the J2 antibody while minimizing non-specific interactions with single-stranded RNA or DNA, supporting the assay's high specificity and robustness for research and diagnostic applications.

The KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA employs the quantitative sandwich enzyme immunoassay technique. It is based on the use of two double-stranded RNA (dsRNA)-specific monoclonal antibodies which allows sensitive and selective detection of dsRNA molecules (>=40 bp), independent of their nucleotide composition and sequence. Antibodies to dsRNA (J2) are pre-coated onto microwells. Samples and standards are pipetted into microwells and are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Anti-dsRNA (K1) is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use 3,3',5,5' Tetra Methyl Benzidine (TMB) substrate solution is added to microwells and color develops proportionally to the amount of dsRNA in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nmValidation Parameters and Acceptance Criteria

1. Double-Stranded RNA (dsRNA) J2 based Values and Recommended ELISA Range

This table summarizes Double-Stranded RNA (dsRNA) J2 levels across different therapies and suggested corresponding ELISA working ranges.

Application	Expected Double-Stranded RNA (dsRNA) J2 Range (ng/ml)	Recommended ELISA Range (ng/ml)
Post low-dose administration (e.g., early-phase studies or priming doses)	5–20	0–50
Standard therapeutic dose (immunostimulatory or adjuvant use)	25–80	0–100
High-dose regimens (oncology or antiviral intensive protocols)	70–150	0–200
Pharmacokinetic studies / systemic delivery evaluation	120–250	0–300

Note: Assay sensitivity <5 ng/mL recommended for baseline detection; upper limit ≥250 ng/mL advised for CRS monitoring.

The KRIBIOLISA Double-Stranded RNA (dsRNA) J2 based ELISA kit is developed using an assay range of 0 - 100 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 32 fold dilution and the values are within the acceptable range.

2. Specificity and Selectivity

2.1 Specificity

The capture and detection antibodies employed in the dsRNA J2-based ELISA are monoclonal antibodies that specifically recognize the A-form double-stranded RNA helix, particularly sequences ≥40 base pairs. The J2 antibody exhibits a high binding affinity to synthetic and naturally occurring dsRNA molecules—such as viral replication intermediates or immunostimulatory RNAs—while showing minimal affinity for single-stranded RNA, DNA, or short duplexes. This specificity enables the assay to detect dsRNA irrespective of sequence, provided structural criteria are met.

2.2 Selectivity

The ELISA demonstrates low to no cross-reactivity with single-stranded RNA, DNA, or structurally unrelated nucleic acid fragments. It also excludes non-dsRNA species such as mRNA, tRNA, or siRNA below the structural threshold for J2 recognition. Additionally, the assay maintains high selectivity in complex biological matrices (e.g., serum, lysate, or viral cultures), with negligible interference from host nucleases, proteins, or other nucleic acid-binding molecules. This ensures consistent and accurate quantification of immunologically relevant dsRNA without false positives.

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 dsRNA J2-based ELISA = 0.3 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 dsRNA J2-based ELISA – 0.5 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:

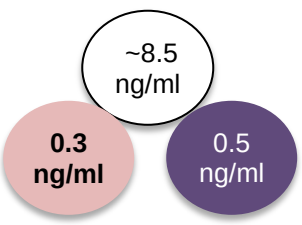
Neutralization ELISA: Quantifies potency of antibodies inhibiting target–ligand interaction.

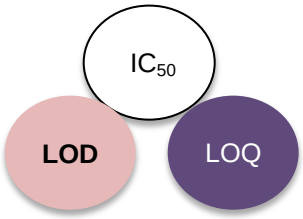
Drug Potency Testing: Measures concentration at which drug inhibits 50% of target activity.

IC_{50} for KRIBIOLISA dsRNA J2-based ELISA = ~8.5 ng/ml

Summary:

Parameter	Value (ng/ml)
LOD	0.3 ng/ml



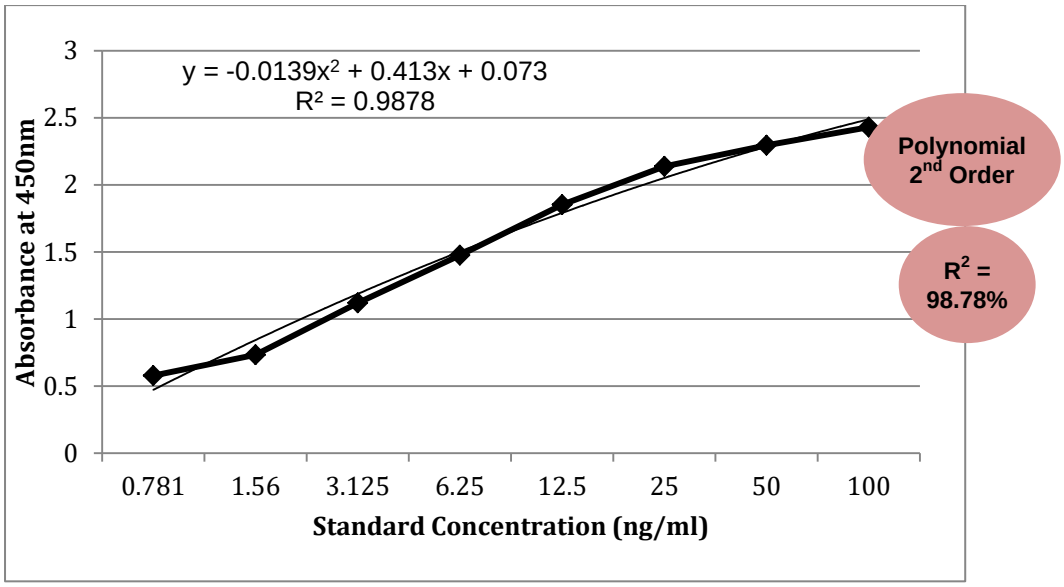


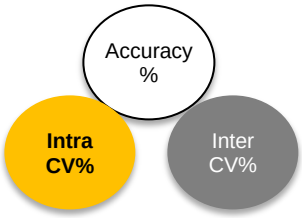
LOQ	0.5 ng/ml
IC_{50}	8.5 ng/ml

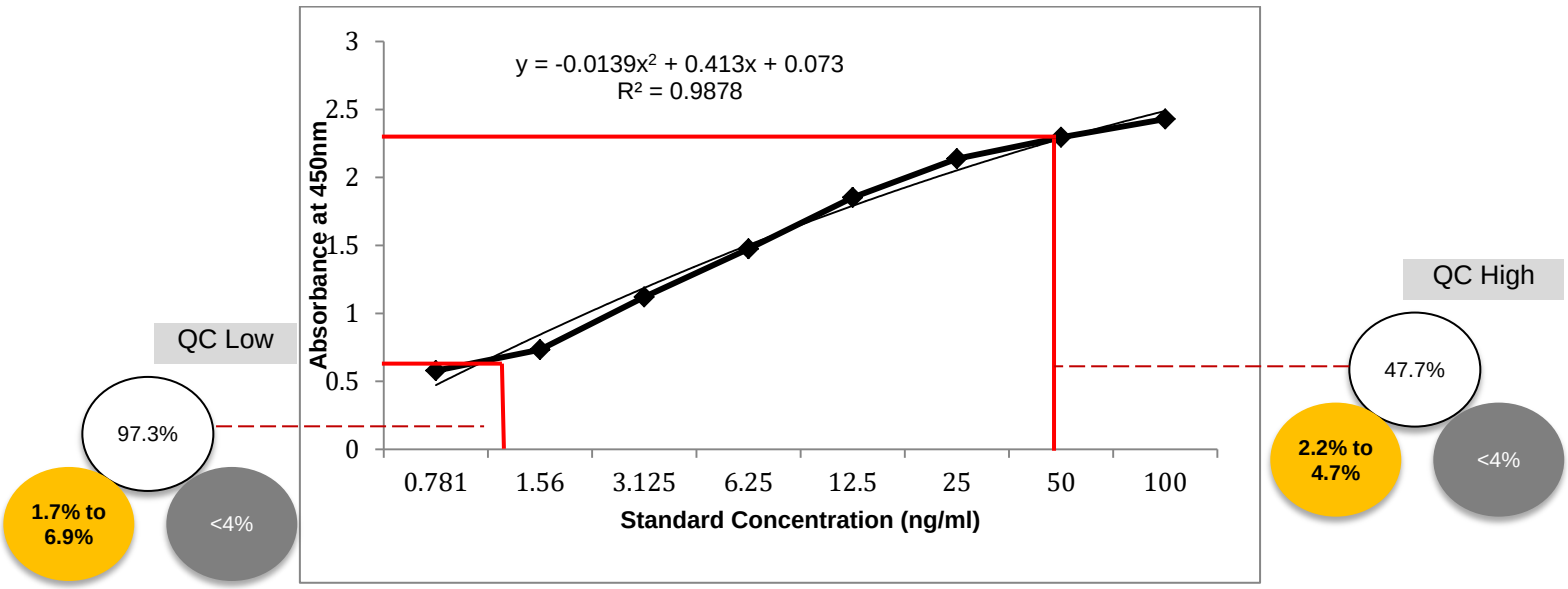
Regulatory Note:
LOD S/N ≥ 3:1, LOQ ≥ 10:1, %CV ≤ 20% **S/N = Signal / Noise Ratio*

3. Linearity and Range

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery
0	--	--	--
0.781	0.578	0.9	115.2
1.56	0.733	1.4	89.7
3.125	1.120	3.2	102.1
6.25	1.475	6.1	97.6
12.5	1.853	12.8	102.4
25	2.138	26.2	104.8
50	2.295	46.3	92.6
100	2.430	101.3	101.3
Positive Control (75 ng/ml)	2.101	23.51	31.4
Low QC Control (1 ng/ml)	0.602	0.97	97.3
High QC Control (50 ng/ml)	2.106	23.85	47.7







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

Precision was assessed by analyzing three standard concentrations (1.56 ng/ml, 25 ng/ml, and 100 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
1.56	1.7% to 6.9%	<6%
25	2.1% to 4.1%	<3%
100	2.2% to 4.7%	<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 dsRNA J2 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 1:1, 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 dsRNA J2 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
1:1	50	2.389	44.9	89.8	111.4
1:2	25	2.125	20.2	80.8	123.8
1:4	12.5	1.902	10.5	83.6	119.6
1:8	6.25	1.612	5.8	92.1	108.6
1:16	3.125	1.302	3.0	97.3	102.8
1:32	1.56	0.912	1.2	77.3	129.5

B) Human Plasma:

Dilution	Expected Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration (ng/ml)	% Recovery	% Deviation
1:1	50	2.502	45.1	90.2	110.9
1:2	25	2.211	20.2	80.6	124.1
1:4	12.5	2.001	12.8	102.7	97.4
1:8	6.25	1.658	6.7	107.7	92.8
1:16	3.125	1.212	3.0	95.3	105.0
1:32	1.56	0.912	1.6	102.1	98.1

Results:

i. Parallelism is generally 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 dsRNA J2 ELISA kit was tested for matrix effect on human serum, plasma and physiological buffer 7.4 to mimic tear fluid samples.

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 KRIBIOLISA dsRNA J2 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 + 1:100 human serum)
- Assay buffer spiked with human serum (buffer + 1:100 human plasma)

Samples were tested across the standard curve range (0–100 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	0.155	0.258	7.28	35.2
0.781	0.578	0.789	14.91	21.8
1.560	0.733	0.934	14.19	17.0
3.125	1.120	1.358	16.86	13.6
6.25	1.475	1.678	14.36	9.1
12.5	1.853	2.002	10.55	5.5
25	2.138	2.225	6.19	2.8
50	2.295	2.478	12.97	5.4
100	2.430	2.689	18.34	7.2

Standard (ng/ml)	Mean Absorbance (Buffer)	Mean Absorbance (Buffer + 1:100 Human Plasma)	% Standard Deviation	% CV
0	0.155	0.189	2.40	14.0
0.781	0.578	0.658	5.65	9.1
1.560	0.733	0.898	11.64	14.3
3.125	1.120	1.229	7.74	6.6
6.25	1.475	1.612	9.70	6.3
12.5	1.853	2.008	10.97	5.7
25	2.138	2.298	11.35	5.1
50	2.295	2.512	15.37	6.4
100	2.430	2.698	18.98	7.4

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 dsRNA J2 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.

7. Sample Handling and Storage Conditions

A) Specimen Collection and Handling:

For prepared solutions: Dilute to expected concentration within the kit assay range using Sample Diluent provided in the kit.

For lyophilized preparations including vaccines: Reconstitute using the Sample Diluent. Keep for 5 mins and mix well. Use the Sample Diluent for further dilutions to bring the sample within the expected assay range of the kit.

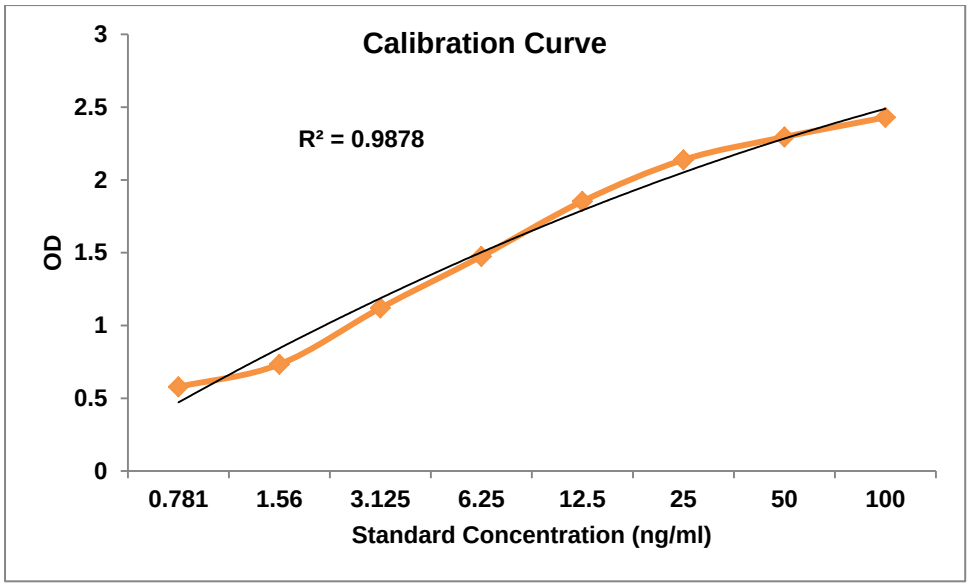
B) Handling / Storage:

- All reagents should be stored at temperature indicated on the labels.
- Upon receiving the kit, prepare small aliquots of dsRNA-specific K1 Detection HRP Conjugate and store it at -20ºC. Avoid repeated freeze and thaw.
- 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.

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

	1:1	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% (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

Bou-Nader, C., Juma, K. M., Bothra, A., Brasington, A. J., Ghirlando, R., Suzuki, M., Garboczi, D. N., Leppla, S. H., & Zhang, J. (2025). Specificity and mechanism of the double-stranded RNA-specific J2 monoclonal antibody. *bioRxiv (Cold Spring Harbor Laboratory)*. https://doi.org/10.1101/2025.05.09.649859

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