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

REF : KBBA56

Ver 2.4

RUO

Enzyme Immunoassay for the Qualitative / Quantitative Screening of double stranded RNA in mRNA based preparations

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

For Research and In-Vitro Test Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Krishgen Biosystems Private Limited is strictly prohibited.

REF KBBA56 **Σ 5 X 96 Tests**

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Introduction:

The J2 anti-dsRNA IgG2a monoclonal antibody (Schönborn et al. 1991) has become the gold standard in dsRNA detection. It was used initially for the study of plant viruses, but since the seminal paper of Weber et al. in 2006, where J2 was used to show that all the positive strand RNA viruses tested produced copious amounts of dsRNA in infected cells, this antibody has been used extensively in a wide range of systems, as documented in over 200 scientific publications.

J2 can be used to detect dsRNA intermediates of viruses as diverse as Hepatitis C virus, Dengue virus, rhinovirus, Chikungunya virus, Rabies virus, Polio virus, Classic swine fever virus, Brome mosaic virus and many more in cultured cells and also in fixed paraffin-embedded histological samples. J2 has been used to elucidate how anti-viral responses are initiated, what counter-strategies viruses have adopted to avoid them, and to explore the viral life cycle by enabling ultrastructural localization studies of viral nucleic acid replication sites (Welsch et al., 2009 & Knoops et al., 2011). J2 has also been recommended as a diagnostic tool to detect whether an unknown pathogen is bacterial or viral in nature (Richardson et al., 2010).

Recently J2 has also been used to monitor the removal of dsRNA from in vitro synthesized mRNA preparations that may have potential use in gene therapy (Kariko et al., 2011). J2 has been used successfully in various immunocapture methods, such as ELISA.

Intended Use:

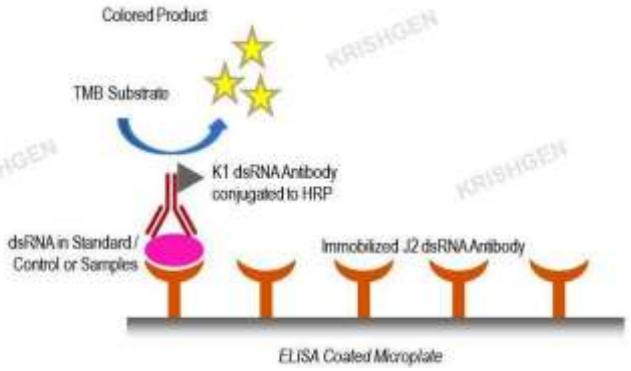
The KRIBIOLISA® Double-Stranded RNA (dsRNA) ELISA is used as an analytical tool for the qualitative / quantitative determination of Double-Stranded RNA (dsRNA) in mRNA based preparations. We recommend using the ELISA to detect viral dsRNAs or large natural or synthetic dsRNAs of non-viral origin in nucleic acid extracts, as well as to detect the presence of undesired dsRNA molecules in artificially synthesized (m)RNA preparations. Serial dilutions of the Poly(I:C) dsRNA standard (included in the kit) can be used as a positive quantitative control.

Note: The Poly(I:C) dsRNA positive control included in this sandwich ELISA kit is not intended to be used as a quantitative standard for other dsRNA preparations. The anti dsRNA antibodies J2 and/or K2 used in this kit may exhibit a different degree of reactivity with different dsRNAs obtained from synthetic or natural sources. It is therefore only intended to be used as a positive quantitative control to see if the ELISA has been executed correctly and that the test shows a linear relationship between the amount of dsRNA and the read out for the absorbance values at 450 nm.

Principle:

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 nm.



Materials Provided:

Part	Description	Qty
Anti-J2 dsRNA Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with J2 antibody to dsRNA	5 x 96 wells
Standard / Control	Poly (I:C) dsRNA in RNAse/DNAse-free, sterile buffer (lyophilized, concentrated 1000 ng/ml)	5 vials
dsRNA-specific K1 Detection:HRP Conjugate	dsRNA-specific K1 conjugated to HRP (concentrated, frozen)	2 vial
(1X) Conjugate Diluent	Buffer with protein stabilizer and preservatives 0.02% methylisothiazolinone and 0.02% bromonitrodioxane	60 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer	2 x 100 ml
(20X) Wash Buffer	20-fold concentrated solution of buffered surfactant with preservative thiomersol < 0.01%. May turn yellow over time	125 ml
TMB Substrate	Stabilized Chromogen	60 ml
Stop Solution	0.73M Phosphoric Acid	60 ml
Instruction Manual		1 no

Materials to be provided by the End-User:

1. Microplate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Use clean, RNase-free micro-centrifuge tubes with cap and pipette tips.
4. Deionized (DI) water.
5. Wash bottle or automated microplate washer.
6. Timer.
7. Absorbent paper

Health Hazard Warnings:

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

Handling / Storage:

1. All reagents should be stored at temperature indicated on the labels.
2. Upon receiving the kit, prepare small aliquots of dsRNA-specific K1 Detection HRP Conjugate and store it at -20ºC. Avoid repeated freeze and thaw.
3. All the reagents and wash solutions should be used within 12 months from manufacturing date.
4. 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.

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.

Reagent Preparation:

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. To make Wash Buffer (1X), dilute 25 ml of Wash Buffer (20X) in 475 ml of DI water.

4. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of (1X) Sample Diluent to obtain a concentration of 1000 ng/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 100 ul of Reconstituted **Standard (1000 ng/ml)** with 900 ul of (1X) Sample Diluent to generate a **100 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the (1X) Sample Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
1000 ng/ml	Reconstituted Standard	Lyophilized Standard provided in the Kit + 1 ml of Sample Diluent (1X)
100 ng/ml	Standard No.8	100 ul Reconstituted Standard (1000 ng/ml) + 900 ul Sample Diluent (1X)
50 ng/ml	Standard No.7	500 ul Standard No.8 + 500 ul Sample Diluent (1X)
25 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Sample Diluent (1X)
12.5 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Sample Diluent (1X)
6.25 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Sample Diluent (1X)
3.125 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Sample Diluent (1X)
1.56 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Sample Diluent (1X)
0.781 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Sample Diluent (1X)
0 ng/ml	Standard No.0	Only Sample Diluent ((1X)

Use the Standards as soon as possible upon reconstitution. Discard balance standards after use.

5. **Positive Control Preparation:** The positive control for qualitative testing may be prepared as above step no. (4) based on the cut-off quantification required in the assay. We recommend making Low Positive Control at 1.56 ng/ml and a High Positive Control at 50 ng/ml for your assays. The Positive Control may be reconstituted and prepared based on the requirements for testing and specifications in each individual laboratory. (Note: The Positive Control is not offered as a separate control of the kit and is to be prepared from the lyophilized standard).
6. **Working dsRNA-specific K1 Detection:HRP Conjugate:** Refer lot specific COA Reagent Preparation sheet for working conjugate diluent.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of dsRNA. High Dose Hook Effect is due to excess of antibody for very high concentrations of dsRNA present in the sample. High Dose Hook effect is most likely encountered from samples early in the purification process. If Hook Effect is possible, the samples to be assayed should be diluted with a compatible diluent. Thus if the dsRNA concentration of the undiluted sample is less than the diluted sample, this may be indicative of the Hook Effect.
3. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of dsRNA.
4. It is recommended that all Controls and Samples be assayed in duplicates.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
7. The plates should be read within 30 minutes after adding the Stop Solution.
8. Make a work list in order to identify the location of Controls and Samples.

Assay Procedure:

1. Bring all reagents to Room Temperature prior to use. It is strongly recommended that all Controls and samples should be run in duplicates or triplicates.

2. Add **100 ul** of prepared **Positive Control/Standards** and **Samples** in their respective wells.

3. Seal the plate and Incubate at **37ºC** for **120 minutes.**

4. Aspirate and wash the plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

5. Add **100 ul** diluted **Working dsRNA-specific K1 Detection:HRP Conjugate** to all the wells.

6. Seal the plate and Incubate at **37ºC** for **60 minutes.**

7. Repeat the wash step (4).

8. Add **100 ul** of **TMB substrate** in each well.

9. Incubate the microplate for 30 minutes at RT in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

10. Pipette out **100 ul** of **Stop Solution**. Wells should turn from blue to yellow in color.

11. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution.

Interpretation of the Results:

For Qualitative Results:
Positive: absorbance of samples > absorbance of Positive Control
Negative: absorbance of samples < absorbance of Positive Control

For Quantitative Results:
Determine the Mean Absorbance for each set of duplicate/triplicate Standards and Samples. Using standard graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown dsRNA concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor.

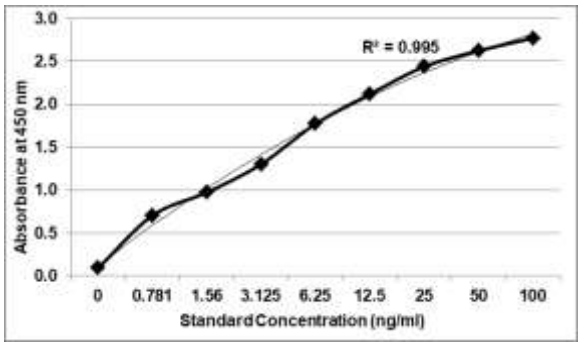
Software which is able to generate a 4 PL (2^{nd} order) or a cubic spline curve-fit is best recommended for automated results.
Note:
i) It is recommended to repeat the assay at a different dilution factor in the following cases:
   - If the sample absorbance value is below the first standard.
   - If the absorbance value is equivalent or higher than the 100 ng/ml standard.
ii) Incase poor recoveries are observed; please contact us at email:sales1@krishgen.com to support further optimization using our alternate sample diluents.

Typical Data (representative only)

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.092	--	--
0.781	0.704	0.8	107.5
1.56	0.974	1.6	99.7
3.125	1.302	2.9	92.2
6.25	1.772	6.5	104.3
12.5	2.117	12.5	99.7
25	2.437	26.4	105.5
50	2.622	48.0	96.0
100	2.770	97.2	97.2

Typical Graph (representative only)

Abs = absorbance at 450 nm



Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

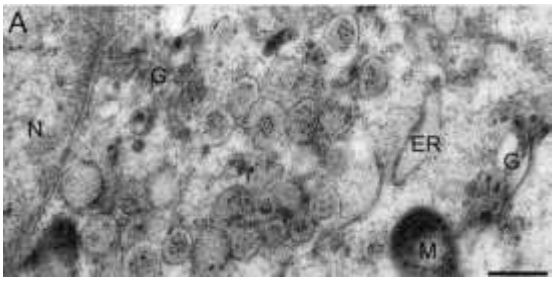
Performance Characteristics of the Kit:

This kit has been validated as per EMA/FDA guidelines in line with ICH Code for Harmonization of Biological Assays and the Assay Guidance Manual.

Sensitivity:
Limit Of Detection: It is defined as the lowest detectable concentration corresponding to a signal of Mean of '0' standard plus 2*SD.
10 replicates of '0' standards were evaluated and the ELISA kit LOD was found to be ~0.25 ng/ml.

Specificity:
This assay works on the important principle of using a capture antibody which is J2 based; IgG2a kappa monoclonal antibody to dsRNA. Anti-dsRNA monoclonal antibody J2 recognizes double-stranded RNA (dsRNA) provided that the length of the helix is greater than or equal to 40 bp. dsRNA-recognition is independent of the sequence and nucleotide composition of the antigen. All naturally occurring dsRNAs investigated up to now (40-50 species) as well as poly(I)-poly(C) and poly(A)-poly(U) have been recognized by Anti-dsRNA monoclonal antibody J2 although in some assays its affinity to poly(I)-poly(C) is about 10 times lower than that to other dsRNA antigens.

Recent publications have indicated J2 based antibody to be used to monitor the removal of dsRNA from in vitro synthethised mRNA preparations that may have potential use in gene therapy (Kariko et al., 2011).



Immune electron microscopy image, where J2 antibody labeling shows abundance of dsRNA (black dots) in double-membrane vesicles (DMVs) of SARS-CoV infected Vero E6 cells, fixed at 7 hours post-infection. J2 was detected using immunogold conjugated to protein A. G: Golgi complex, M: mitochondria, N: nucleus. Figure taken from Knoops et al. (2008) PLoS Biol 6:e226.

The control / standard provided in the kit is a low molecular weight Poly (I:C) dsRNA which is intended to be used as a positive control in double-stranded (ds)RNA detection. The sequence does not contain any viral or potentially transforming elements. The Positive Control maybe used for dsRNA detection by anti dsRNA monoclonal J2, J5, K1 and K2 antibodies. The anti dsRNA antibodies may exhibit a different degree of reactivity with different dsRNAs either produced synthetically or obtained from natural sources. It is therefore only intended to be used as a positive control to see if the ELISA has been executed correctly. The dilution of the control / standard shown along with the typical data and typical graph is to demonstrate a linear relationship between the amount of dsRNA and the read out at OD450 in the 4-parameter analysis. It cannot be used to determine the concentration of a different type of dsRNA.

Precision:
Precision is defined as the percent coefficient of variation (%CV) i.e. standard deviation divided by the mean and multiplied by 100. Assay precision was determined by both intra (n=5 assays) and inter assay (n=5 assays) reproducibility on two pools with low (0.781 ng/ml), medium (12.5 ng/ml) and high (100 ng/ml) concentrations.

While actual precision may vary from laboratory to laboratory and technician to technician, it is recommended that all operators achieve precision below these design goals before reporting results.

Pool	Intra Assay %CV	Intra Assay %CV
Low	<12%	<15%
Medium	<12%	<12%
High	<10%	<10%

Lot to Lot Variation Controlled in Production:
We do continuous monitoring of the kits produced in the lots/batches and the %CV of the last 3 lots manufactured by us is indicated herein below. The internal QC parameter for our production is to ensure that each lot manufactured is within the parameter of 10%CV.

	Lot A	Lot B	Lot C	Lot D				
Standard Concentration (ng/ml)	Abs OD450	Abs OD450	Abs OD450	Abs OD450	Mean Abs	%STD DEV	CV	%CV
0	0.091	0.189	0.179	0.100	0.140	---	---	---
0.781	0.573	0.481	0.399	0.512	0.491	6.3	0.15	14.7
1.56	0.74	0.664	0.698	0.713	0.704	2.8	0.05	4.5
3.125	1.021	1.052	0.859	1.088	1.005	8.7	0.10	10.0
6.25	1.485	1.512	1.359	1.613	1.492	9.0	0.07	7.0
12.5	1.86	1.998	1.848	2.054	1.940	8.8	0.05	5.3
25	2.255	2.387	2.356	2.418	2.354	6.1	0.03	3.0
50	2.7	2.623	2.649	2.665	2.659	2.8	0.01	1.2
100	2.888	2.875	2.885	2.825	2.868	2.5	0.01	1.0

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (<0.1% w/v) as preservative. They must not beswallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **porcine fluids** or organs used in the preparation of this kit. Therefore, handleall components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves
  - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.


References:

Detection of double-stranded RNA by ELISA and dot immunobinding assay using an antiserum to synthetic polynucleotides
J Aramburu, J Navas-Castillo, P Moreno… - Journal of virological …, 1991 - Elsevier

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M Bokarewa, A Tarkowski, M Lind… - European journal of …, 2008 - Wiley Online Library

Monoclonal antibodies to double-stranded RNA as probes of RNA structure in crude nucleic acid extracts
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Detection of Double-Stranded RNA (DSRNA) in Crude Extracts of Virus-Infected Plants by Indirect ELISA
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Determination of antibodies to double-stranded RNA by enzyme-linked immunosorbent assay (ELISA)
AK Field, ME Davies, AA Tytell - Proceedings of the society …, 1980 - journals.sagepub.com

Preparation and characterization of monoclonal antibodies to double-stranded RNA
C Powell - Phytopathology, 1991 - apsnet.org

Detection of plum pox virus by isolation of double-stranded ribonucleic acid (dsRNA)
E Maiss, E Breyel, R Casper, DE Lesemann - EPPO Bulletin, 1987 - Wiley Online Library

dsRNA-induced expression of thymic stromal lymphopoietin (TSLP) in asthmatic epithelial cells is inhibited by a small airway relaxant
A Brandelius, Y Yudina, J Calvén, L Bjermer… - Pulmonary …, 2011 - Elsevier

SCHEMATIC ASSAY PROCEDURE

1. Remove all components, 30 minutes before adding into the assay plate.

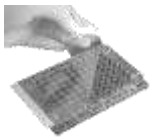
Components NOW (2-8ºC) → 30 mins RT Before Use → 

→ Thaw at Room Temperature (18-24ºC) → USE

2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.

Kit Components RESULTS → **AVOID** → **COOL (2-8ºC)** → **THAW (22±4ºC)** → **THAW (22±4ºC)** → **COOL (2-8ºC)** → **AVOID** → NO CHANGE IN

3.  Pipette **100 ul** prepared **Standards** / **Samples** into each well.

4. **Cover plate**  **and incubate for** 

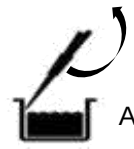
at 37°C.

5.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

6.  Pipette **100 ul** of **diluted Working dsRNA-specific K1 Detection:HRP Conjugate** into each well.

7. Cover plate  and incubate for 

at 37°C.

8.  Aspirate and wash wells 4 times with **Wash Buffer (1X).**

9.  Pipette **100 ul TMB Substrate** into each well.

10. Cover plate  and incubate for 

at RT.

11.  Pipette **100 ul Stop Solution** into each well.

12. Read absorbance at 450 nm with a  microplate reader within 

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450 nm	Mean Absorbance	ng/ml dsRNA equivalent
1A 2A	zero std zero std			
1B 2B	0.781 ng/ml 0.781 ng/ml			
1C 2C	1.56 ng/ml 1.56 ng/ml			
1D 2D	3.125 ng/ml 3.125 ng/ml			
1E 2E	6.25 ng/ml 6.25 ng/ml			
1F 2F	12.5 ng/ml 12.5 ng/ml			
1G 2G	25 ng/ml 25 ng/ml			
1H 2H	50 ng/ml 50 ng/ml			
3A 4A	100 ng/ml 100 ng/ml			
3B 4B	Sample Sample			

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

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THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	**Anti-J2 dsRNA Coated Microtiter Plate (12x8 wells)**
STD	**dsRNA Standard, Lyophilized**
HRP CONJ	**dsRNA-specific K1 Detection:HRP Conjugate (concentrated)**
1X CONJ DIL	**(1X) Conjugate Diluent**
1X SAMP DIL	**(1X) Sample Diluent**
20X WASH BUF	**(20X) Wash Buffer**
SUB TMB	**TMB Substrate**
SOLN STOP	**Stop Solution**
	**Consult Instructions for Use**
REF	**Catalog Number**
	**Expiration Date**
	**Storage Temperature**