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EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com info@eaglebio.com

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

REF: KBBA56

Ver1.2

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**

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REF KBBA56 **5 X 96 tests**

KRISHGEN BioSystems | For US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005
For Asia / India: tel: +91(22)-49198700
Email: sales@krishgen.com

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 cylce by enabling ultrastructiural localisation 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 unkown 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 synthethised 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:

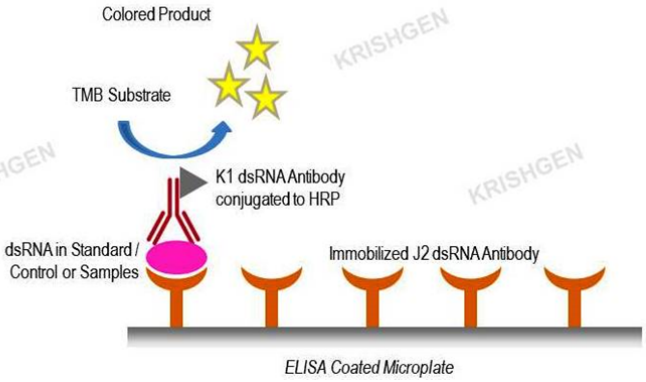
The KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA is used as an analytical tool for the qualitative / quantitative determination of Double-Stranded RNA (dsRNA) in mRNA based preparations. We recommend using the ELISA to detect viral dsRNAs or large natural or synthetic dsRNAs of non-viral origin in nucleic 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 450nm.

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 substrate solution (TMB) 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	HRP conjugated dsRNA-specific K1 Detection Antibody prepared in buffer with 1% protein stabilizer, ready-to-use	60 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer	200 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	2N Sulfuric 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. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. 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 25ml of Wash Buffer (20X) in 475 ml of DI water.
4. **Standards Preparation**: Reconstitute the concentrated Standard lyophilized vial with 1 ml of Standard Diluent to obtain a concentration of 1000 ng/ml. Keep the vial for 15 mins with gentle agitation before making further dilutions. Dilute 200 ul of original **Standard (1000 ng/ml)** with 800 ul of Standard Diluent to generate a **200 ng/ml Standard Solution**. Prepare further **Standards** by serially diluting the Standard Solution as per the below table. Use the Standard Diluent as the Zero Standard (Standard No.0).

Standard Concentration	Standard Vial	Dilution Particulars
1000 ng/ml	Original Standard	Original Standard provided in the Kit + Reconstituted in 1 ml of Standard Diluent
200 ng/ml	Standard No.7	200 ul Original Standard (1000 ng/ml) + 800 ul Standard Diluent
100 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
50 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
25 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
12.5 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
6.25 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
3.125 ng/ml	Standard No.1	500 ul Standard No.2 + 500 ul Standard Diluent
0 ng/ml	Standard No.0	Only Standard Diluent

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 6.25 ng/ml and a High Positive Control at 100 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.

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** to **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 reader.

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 450nm) 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.

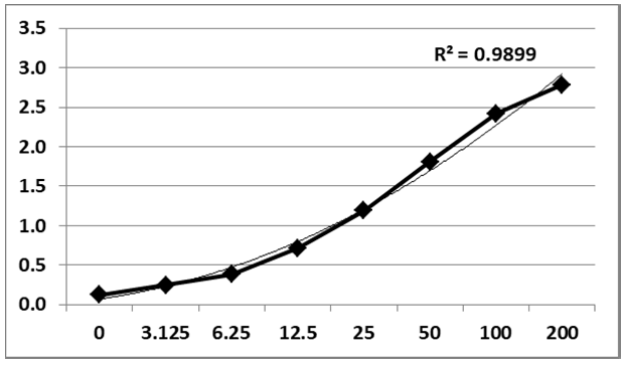
Software which is able to generate a 4 PL (2nd order) or a cubic spline curve-fit is best recommended for automated results.

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 200 ng/ml standard.
ii) Incase poor recoveries are observed; please contact us at email:sales@krishgen.com to support further optimization using our alternate sample diluents.

Typical Data

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.124	0.125	0.124	0.2	--
3.125	0.290	0.208	0.249	3.1	99.6
6.25	0.423	0.342	0.382	5.8	93.4
12.5	0.771	0.660	0.715	12.9	103.0
25	1.198	1.188	1.193	25.2	100.6
50	1.778	1.8	1.807	49.3	98.7
100	2.368	2.477	2.422	100.8	100.8
200	2.725	2.844	2.784	199.6	99.8

Typical Graph



Abs = absorbance at 450nm

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 1.5 ng/ml

Specificity:
This assay works on the sandwich ELISA principle and uses the J2 (IgG2a kappa) mouse monoclonal antibody to dsRNA as the capture antibody. 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.

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 (31.25 ng/ml), medium (250 ng/ml) and high (2000 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	Inter Assay %CV
Low	<12%	<12%
Medium	<12%	<12%
High	<10%	<10%

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/w) 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
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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)
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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.

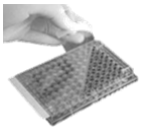
Components (2-8ºC) → 30 mins RT Before Use → 

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

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

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

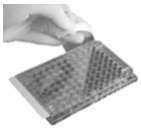
3.  Pipette **100 ul** prepared **Standards** / **Samples** into each well.

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

at 37°C.

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

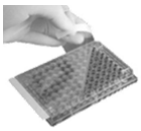
6.  Pipette **100 ul** of **dsRNA-specific K1 Detection:HRP Conjugate** into each well.

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 37°C.

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

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

of stopping reaction.

Typical Example of a Work List

Well #	Contents	Absorbance at 450nm	Mean Absorbance	ng/ml dsRNA equivalent
1A 2A	zero std zero std			
1B 2B	3.125 ng/ml 3.125 ng/ml			
1C 2C	6.25 ng/ml 6.25 ng/ml			
1D 2D	12.5 ng/ml 12.5 ng/ml			
1E 2E	25 ng/ml 25 ng/ml			
1F 2F	50 ng/ml 50 ng/ml			
1G 2G	100 ng/ml 100 ng/ml			
1H 2H	200 ng/ml 200 ng/ml			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

LIMITED WARRANTY

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SYMBOLS KEY

MTP	**Anti-J2 dsRNA Coated Microtiter Plate (12x8 wells)**
STD	**dsRNA Standard, lyophilized**
HRP CONJ	**Conjugate Horseradish Peroxidase**
1X STD DIL	**(1X) Standard 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**