KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA (K1 based)

REF : KBBA57

Ver2.1

RUO

Enzyme Immunoassay for the Qualitative / Semi-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 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 is strictly prohibited.

REF KBBA57  **5 X 96 tests**

 **KRISHGEN BioSystems |** For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: tel +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgen.com

Introduction:

The Double-stranded RNA (dsRNA) ELISA kit (K1 based) can be used 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 pharmaceutical preparations.

The K1 monoclonal antibody recognizes dsRNA with similar affinity to widely used J2 antibody. It can be used for the histological and cytological detection of dsRNA in cells and tissues. It has proven especially useful as an alternative to J2 to resolve cross-reactions and/or remove unwanted background, in those rare experimental setups where J2 did not provide satisfactory results. K1 can be used to detect dsRNA intermediates of viruses as diverse as Hepatitis virus, Theiler's murine encephalomyelitis virus or Japanese encephalitis virus. It has been for the detection of dsRNA in cultured cells and in fixed paraffin-embedded histological samples. as documented in various publications.

Intended Use:

The KRIBIOLISA™ Double-Stranded RNA (dsRNA) ELISA is used as an analytical tool for the qualitative / semi-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. Antibodies to dsRNA (K1) are pre-coated onto microwells. Samples and standards are pipetted into microwells along with Detection Anti-dsRNA (K2) and dsRNA in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated Anti-dsRNA (K2) antibody 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-K1 dsRNA Coated Microtiter Plate	96 well polystyrene microplate (12 strips of 8 wells) coated with K1 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
(1X) Standard Diluent	Buffer with 1% protein stabilizer, ready-to-use	60 ml
dsRNA-specific K2 Detection :HRP Conjugate	dsRNA-specific K2 Detection Antibody prepared in buffer with 1% protein stabilizer, ready-to-use	60 ml
(1X) Sample Diluent	Buffered protein base with protein stabilizer and 0.1% BSA	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 1000ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Timer.
6. 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 640 ul of original **Standard (1000 ng/ml)** with 360 ul of Standard Diluent to generate a **640 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
640 ng/ml	Standard No.7	640 ul Original Standard (1000 ng/ml) + 360 ul Standard Diluent
320 ng/ml	Standard No.6	500 ul Standard No.7 + 500 ul Standard Diluent
160 ng/ml	Standard No.5	500 ul Standard No.6 + 500 ul Standard Diluent
80 ng/ml	Standard No.4	500 ul Standard No.5 + 500 ul Standard Diluent
40 ng/ml	Standard No.3	500 ul Standard No.4 + 500 ul Standard Diluent
20 ng/ml	Standard No.2	500 ul Standard No.3 + 500 ul Standard Diluent
10 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 20 ng/ml and a High Positive Control at 320 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).

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 K2 Detection: HRP Conjugate** to all the wells.

6. Seal the plate and Incubate at **37ºC** for **120 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 Semi-Quantitative Results:
Determine the Mean Absorbance for each set of duplicate 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 polynomial regression (2nd order) or a cubic spline curve-fit is best recommended for automated results.

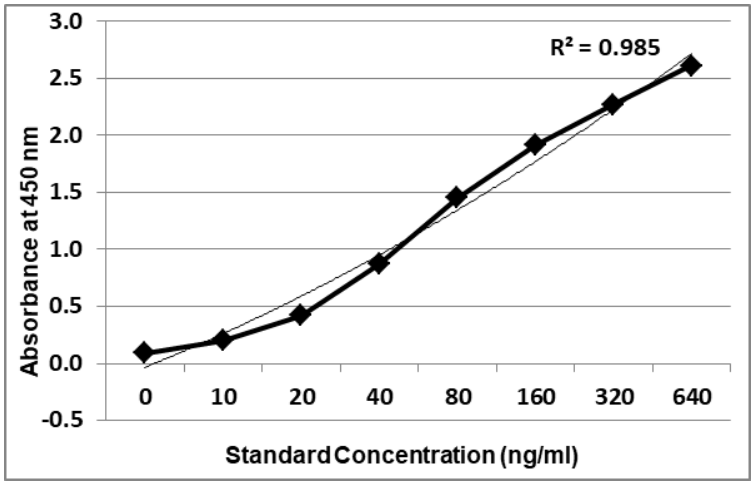
Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the 640 ng/ml standard.

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)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.089	--	--
10	0.199	10.8	107.8
20	0.422	18.6	92.8
40	0.871	39.3	98.2
80	1.454	85.1	106.4
160	1.915	159.4	99.6
320	2.267	288.3	90.1
640	2.611	716.5	112.0

Typical Graph



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 ~6 ng/ml

Specificity:
This assay works on the sandwich ELISA principle and uses the K1 (kappa) mouse monoclonal antibody to dsRNA as the capture antibody. Anti-dsRNA monoclonal antibody K1 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 K1 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 (10 ng/ml), medium (160 ng/ml) and high (640 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.

SCHEMATIC ASSAY PROCEDURE

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

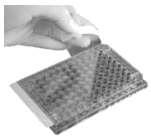
Components (2-8ºC) → 30 mins RT Before Use → 

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

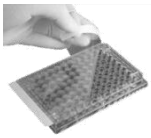
3.  Pipette **100 ul** prepared **Standards** / **Samples** into each well.

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

at 37°C.

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

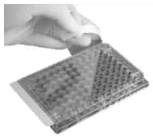
6.  Pipette **100 ul** of **dsRNA-specific K2 Detection :HRP Conjugate** into each well.

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 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	10 ng/ml 10 ng/ml			
1C 2C	20 ng/ml 20 ng/ml			
1D 2D	40 ng/ml 40 ng/ml			
1E 2E	80 ng/ml 80 ng/ml			
1F 2F	160 ng/ml 160 ng/ml			
1G 2G	320 ng/ml 320 ng/ml			
1H 2H	640 ng/ml 640 ng/ml			
3A 4A	Sample Sample			
3B 4B	Sample Sample			

LIMITED WARRANTY

Krishgen Biosystems 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, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems 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 pursuantto written authorization by Krishgen Biosystems.

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

This Limited Warranty states the entire obligation of Krishgen Biosystems 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.

Krishgen Biosystems. 2024

THANK YOU FOR USING KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS®, GENLISA®, DHARMAPLEX™, GENBULK™, GENLISA™, KRISHZYME®, KRISHGEN®, KRIBIOLISA®, KRISHPLEX®, TITANIUM®, QUALICHEK® are registered trademarks of KRISHGEN BIOSYSTEMS. ©KRISHGEN BIOSYSTEMS. ALL RIGHTS RESERVED.

KRISHGEN BIOSYSTEMS | OUR REAGENTS | YOUR RESEARCH |

SYMBOLS KEY

MTP	**Anti-K1 dsRNA Coated Microtiter Plate (12x8 wells)**
STD	**dsRNA Standard, lyophilized**
HRP CONJ	**Conjugate Horseradish Peroxidase**
1X SAMP DIL	**(1X) Sample Diluent**
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**