KRIBIOLISA® FastNuclease Endonuclease *Serratia Marcescens* ELISA

REF : KBBA36

Ver 3.2

RUO

ELISA for Quantitative Determination of Serratia endonuclease in Cell Culture and Biological Samples

RUO	For Research Use Only	REF	Catalog Number
	St At	LOT	Batch Code
	Ma ctured By		Bio al Risk
	E Date		Co Operating Instructions

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REF KBBA36 Σ 96 tests

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Introduction:

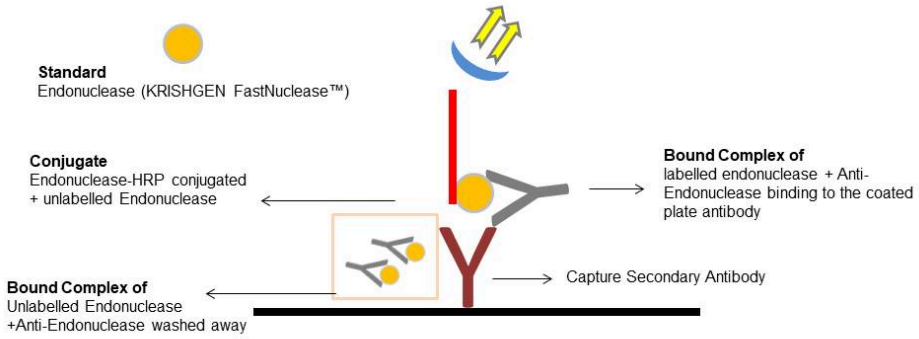
Serratia marcescens nuclease originates from Gram negative bacteria *S. marcescens* and heads a family of homological non-specific nucleases that are widely spread in the world. Serratia nuclease is most studied one and it is capable to cleavage both RNA and DNA in either single or double stranded form.

Intended Use:

The KRIBIOLISA® FastNuclease Endonuclease *Serratia Marcescens* ELISA kit developed for quantitative determination of endonuclease in Cell Culture and Biological Samples from downstream processing where endonuclease is used as a process or purification aid.

Principle:

The FastNuclease Endonuclease *Serratia marcescens* ELISA is a competitive immunoassay for the determination of Serratia endonuclease in sample. The secondary antibodies specific to anti-endonuclease are coated on to the microtiter plate. A constant concentration of HRP labeled endonuclease and varying concentration of standard or sample containing endonuclease compete for binding to anti-endonuclease antibodies. This immune complex is captured by the coated secondary antibody. After incubation and washing, the unbound labeled enzyme is removed. Then addition of substrate develops blue color during incubation period and the reaction is stopped after the addition of stop solution with development of yellow color. The intensity of the color generated is inversely proportional to the amount of endonuclease in the sample.



Materials Provided:

1. Capture Secondary Antibody Microtitre coated well plate (1 x 96 wells) - 1 no
2. Standard (concentrated, 10 ug/ml) – 50 ul
3. Endonuclease:HRP Conjugate - 6 ml
4. Anti-Endonuclease Antibody - 1 vial
5. (20X) Wash Buffer - 25 ml
6. Assay Diluent - 60 ml
7. TMB Substrate - 12 ml
8. Stop Solution - 12 ml
9. Instruction manual

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. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Handling/Storage:

1. It is advisable to aliquot and store the Standard and Detection Antibody at -20°C upon receipt. Rest of the kit components should be stored at 2-8°C.
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.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Sample Preparation:

The test sample should be diluted at least 1:2 in Assay diluent. At this dilution Assay diluent interference in assay will be negligible.

Reagent Preparation (all reagents should be diluted immediately prior to use):

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 20X Wash Buffer in 475 ml of DI water.
4. **Standards Preparation:** Thaw the Concentrated Standard vial provided in the Kit which is of 10 ug/ml. Dilute 10 ul of original **Standard (10 ug/ml)** with 90 ul of Assay Diluent to generate a **1 ug/ml Middle Stock**. Prepare further **Standards** by diluting the Middle Stock as per the below table. Use the Assay Diluent as the Zero Standard (Standard No. 0).

Standard Concentration	Standard Vial	Dilution Particulars
1 ug/ml	Middle Stock	10 ul Original Standard + 90 ul Assay Diluent
256 ng/ml	Standard No.6	64 ul Middle stock + 186 ul Assay Diluent
128 ng/ml	Standard No.5	100 ul Standard No.6 + 100 ul Assay Diluent
64 ng/ml	Standard No.4	100 ul Standard No.5 + 100 ul Assay Diluent
32 ng/ml	Standard No.3	100 ul Standard No.4 + 100 ul Assay Diluent
16 ng/ml	Standard No.2	100 ul Standard No.3 + 100 ul Assay Diluent
8 ng/ml	Standard No.1	100 ul Standard No.2 + 100 ul Assay Diluent
0 ng/ml	Standard No.0	Only Assay Diluent

Use the Standards immediately upon Diluted. Discard balance standard after use. Do not store them for further experiments.

5. **Working Anti-Endonuclease Antibody – Refer to the Reagent Preparation sheet attached with the IFU and COA (enclosed in the kit).**

Assay Procedure:

1. Add It is strongly recommended that all Controls and Samples be run in duplicates or triplicates. A standard curve is required for each assay. All steps must be performed at 37°C.

2. Pipette out **50 ul** of **Standards** or **Samples** in each well Add **50 ul** of **Endonuclease:HRP conjugate** and **50 ul Working Anti-Endonuclease Antibody** to each well. Seal the microplate with the cover membrane, and **incubate at 37°C for 3 hours**.

3. Aspirate and wash plate 5 times and soaking time 1 minute with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

4. Add **100 ul of TMB** solution to each well.

5. Incubate the plate at 37°C for 10 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.

6. Stop reaction by adding **100 ul of Stop Solution** to each well. Positive wells should turn from blue to yellow.

7. Read absorbance at **450 nm** within 10-15 minutes after addition of Stop solution.

Calculation of Results:

Determine the mean absorbance for each set of duplicate standards and samples. Plot the standard curve on graph paper, with concentration on the x-axis and absorbance on the y-axis. Draw the best fit straight line through the standard points. To determine the unknown concentrations, find the unknowns 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.

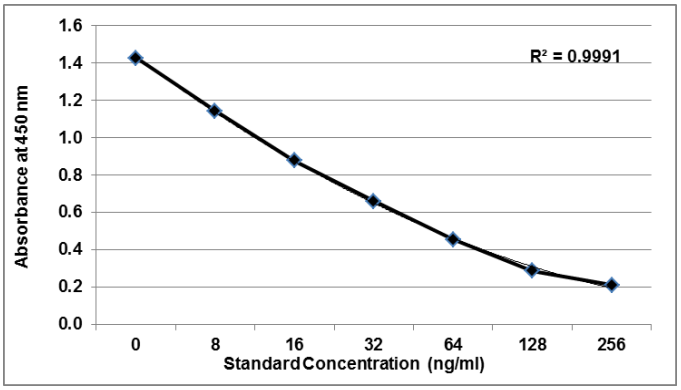
Software which is able to generate a cubic spline curve-fit or a polynomial curve (2nd order) is best recommended for automated results.

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 256 ng/ml standard.

Typical Data (representative only)

Standards provided (ng/ml)	Abs A	Abs B	Mean Abs	% Standard Deviation	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	1.377	1.476	1.426	7.0	--	--
8	1.130	1.159	0.144	2.0	7.4	92.0
16	0.968	0.788	0.878	12.7	17.3	108.4
32	0.665	0.657	0.661	0.6	31.6	98.9
64	0.459	0.455	0.457	0.3	60.2	94.1
128	0.288	0.285	0.287	0.2	134.7	105.2
256	0.224	0.199	0.211	1.8	260.9	101.9

Typical Graph (representative only)



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.

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 be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from **human body fluids** or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all 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

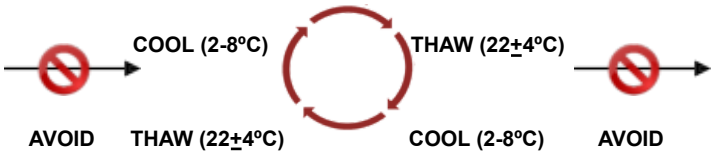
SCHEMATIC ASSAY PROCEDURE

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

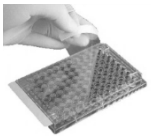
2. Avoid repeated cool-thaw of the components as there will be a loss of activity and this can affect the results.



3. Pipette **50 ul Standards** / **Samples** into each well.

4 Pipette **50 ul Endonuclease:HRP conjugate** into each well.

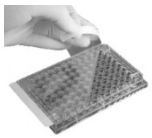
5. Pipette **50 ul Working Anti-Endonuclease Antibody** into each well.

6. Cover plate  and incubate for 

at 37°C.

7. Aspirate and wash wells 5 times with 1 Min soak **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 450 nm with a  microplate reader within 

of stopping reaction.

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SYMBOLS **KEY**

MTP	re Secondary Antibody Microtiter d well plate (12x8 wells)
STD	ard, Concentrated
HRP CONJ	lease:HRP Conjugate
AB	Endonuclease Antibody
ASY DIL	iluent
20X WASH BUF	Buffer
SUB TMB	ostrate
SOLN STOP	tion
	Consult Instructions for Use
REF	g Number
	Expiration Date
	Storage Temperature