KRISHGEN BioSystems

VALIDATION DATA OF FastNuclease™ Endonuclease Serratia Marcescens ELISA (Cat No#KBBA36)

This validation protocol has been adopted in line with the Methodology and Analytical Procedures Guideline recommended by FDA (guidelines May 2018).

Document History

First Codification	History	Date
Version#3.0	**FastNuclease™ Endonuclease Serratia Marcescens ELISA (Catalog No. KBBA36)**	1st January 2022
Version#2.0	**FastNuclease™ Endonuclease Serratia Marcescens ELISA (Catalog No. KBBA36)**	18th November 2021

Approved: Quality Control	Approved: Product Development
Ankita G.	Atul G.

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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.

The Endonuclease Serratia marcescens ELISA kit developed for quantitative determination of endonuclease in samples from downstream processing where endonuclease is used as a process or purification aid.

Validation characteristics considered by us in accordance with the guidelines are listed below:

- **Limit Of Detection**
- **Specificity**
- **Comparison Runs and Data**

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 a preview of the assay and the characteristics of the kit and is generic in nature. We 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 sales@krishgen.com.

Validation Information

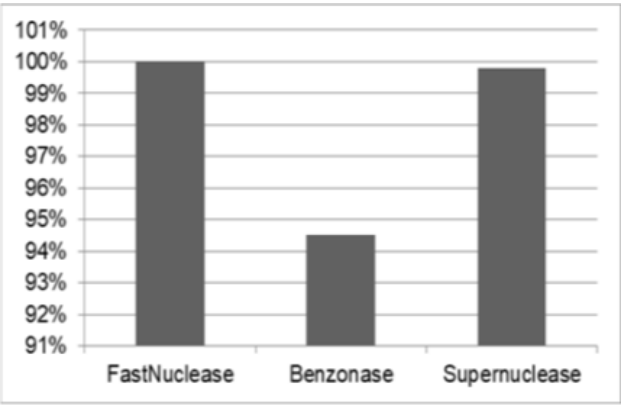
1. Sensitivity:

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 LOD was found to be ~0.1 ng/ml

Limit of Quantification: LoQ is the lowest concentration at which the analyte can not only be reliably detected but at which some predefined goals for bias and imprecision are met. The LoQ was found to be ~0.5 ng/ml.

2. Specificity:

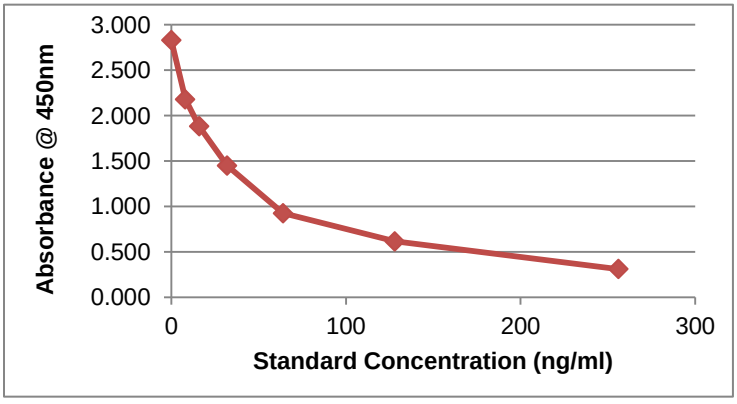
The antibodies used in the kit for capture and detection are specific for Endonuclease Serratia Marcescens. The kit has also been validated using the endonuclease enzyme from Krishgen Biosystems (FastNuclease™), Merck (Benzonase™) and Sino Biologicals (Supernuclease™). The kit has shown efficient recoveries between 86%-118% on buffer spiked with these enzymes.



3. Comparator Between Previous Version of Kit and New Version of the Kit:

Validation using old standard range 8-256 ng/ml as per previous version of the Kit.

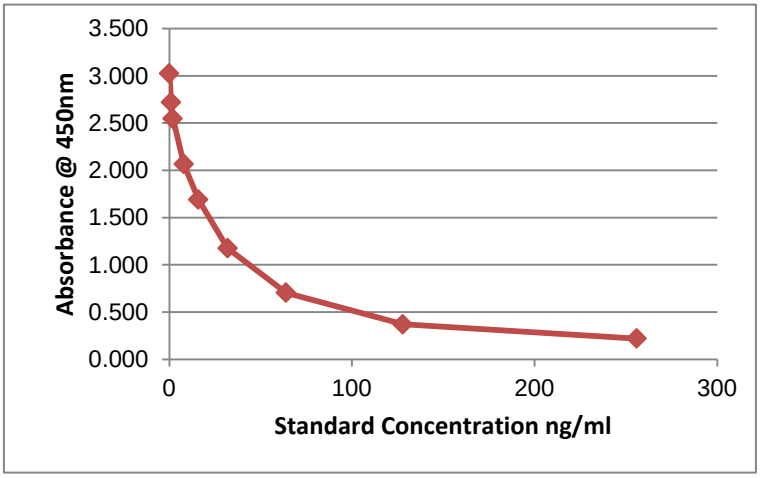
Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	2.713	2.942	2.827	--	--
8	2.177	2.181	2.179	8.5	106.7
16	1.902	1.860	1.881	15.3	95.5
32	1.466	1.429	1.447	30.8	96.1
64	0.999	0.851	0.925	69.5	108.6
128	0.628	0.604	0.616	121.9	95.2
256	0.322	0.298	0.310	257.6	100.6



Data Reduction done using 4PL (2nd order)
Graph Equation =
Regresssion Coefficient (R2) =

Validation using new sensitive standard range 0, 1, 2-256 ng/ml as per new version of the Kit.

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	3.173	2.875	3.024	--	--
1	2.740	2.694	2.717	1.2	121.0
2	2.530	2.566	2.548	2.4	119.8
8	2.127	2.005	2.066	7.6	95.2
16	1.710	1.669	1.690	14.5	90.6
32	1.219	1.130	1.174	32.2	100.6
64	0.706	0.703	0.705	70.2	109.6
128	0.382	0.357	0.370	142.0	111.0
256	0.221	0.217	0.219	215.4	84.1



Data Reduction done using 4PL (2nd order)
Graph Equation =
Regresssion Coefficient (R2) =

Comparison between kit (ver2.0) and new sensitive kit (ver3.0) using standards as below:

New Version Standards in the kit (ng/ml)	Old Version Standards in the kit (ng/ml)
0	--
1	--
2	--
8	8
16	16
32	32
64	64
128	128
256	256

4. Precision:

4.1. Inter/Intra Assay Precision:

Six samples were measured with two replicates and two runs per day for 2 days (n = 8). Samples were measured using one lot of reagent. All data met our acceptance criteria for %CV and 95% confidence intervals for %CV.

Sample	Within-Run		Between-Run		Between-Day		Total	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.21	2.81	0.21	4.28	0.28	2.36	0.71	9.45
2	0.57	3.96	0.78	2.46	0.78	3.42	2.12	9.84
3	1.34	3.72	2.05	2.58	2.12	3.14	5.52	9.44
4	1.34	1.36	2.36	2.33	3.25	3.16	6.96	6.85
5*	1.77	2.83	1.43	2.26	2.83	4.42	6.02	9.51
6*	2.26	3.07	2.57	3.46	2.83	2.38	7.67	8.91
**Samples were tested in the lab that is different from the first four samples with a different operator*								

4.2. Dilutional Linearity:

Linearity: In these studies dilutions were prepared by diluting three samples spiked with Endonuclease. Each dilution was measured in three replicates. Results are summarized in the following table:

Sample	Original Concentration (ng/ml)	Diluted To (ng/ml)	Interpolated Concentration (ng/ml)	% Recovery
Sample 1	10^6	100	99.2	99.2
Sample 2	10^6	100	98.0	98.0
Sample 3	10^6	100	102.08	102.08

5. Traceability and Stability:

5.1 Traceability:

There are no reference standards for Protein L Ligand. The results are reported in ng/mL

5.2 Kit Stability:

Shelf-Life Stability: An accelerated stability study set the shelf-life stability of KRIBIOLISA™ Endonuclease Marsecens ELISA at 12 months for the kit with all components.
Open-Vial Stability: The assay reagents can be stored opened at 2–8°C for up to 8 weeks.

6. High Dose Hook Effect:

Hook effect was measured using two human serum samples spiked with Endonuclease to 1000 ng/ml. Each sample was then diluted with Sample Diluent to a level below the lower limit of the analytical measuring range and measured with a single replicate using one lot of reagent. The data demonstrated the assay is not susceptible to Endonuclease excess up to a concentration of 600 ng/ml.

The high dose hook effect refers to measured levels of antigen displaying a significantly lower absorbance than the actual level present in a sample. This appears when a simultaneous ELISA assay is saturated by a very high concentration of sample antigen binding to all available sites on both the solid phase antibody as well as the detection antibody and thereby preventing the sandwich-formation. The antigen-saturated detection antibodies in solution will be washed off giving a falsely low signal. A “hook” is observed in the curve when data is plotted as a signal versus antigen concentration.

The hook capacity yielding an absorbance reading less than the 256 ng/ml standard was 512 mg/ml.

Conclusion:

The FastNuclease™ Endonuclease Serratia Marcescens has been validated and the standards of the kit are expanded to make it as a high sensitive kit. The sensitivity of the older version and the new version of the kit is 4.5 ng/ml and 0.5 ng/ml respectively.