KRISHGEN BioSystems

VALIDATION DATA OF KRIBIOLISA™ Endonuclease *Serratia marcescens* ELISA (Cat No#KBBA36)

Version #1 dated 23.06.2018

Preliminary Notes:

KRISHGEN KRIBIOLISA™ ELISA kits are routinely being used for analysis of quality of different therapeutic drugs.

Performance Characteristics:

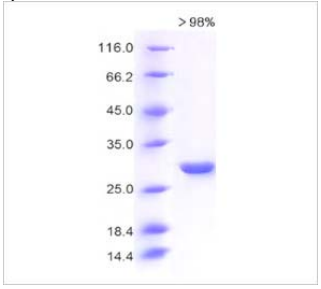
Please note that this validation is performed in our laboratory and will not necessarily be duplicated in your laboratory. This data has been generated to enable the user to get a preview of the assay and the characteristics of the kit and is generic in nature. We recommend that the user performs at 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.

1. **Sensitivity:**

a) **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 1.0 ng/ml

b) **Limit of Quantitation:** It is defined as the lowest concentration for which Coefficient of Variation is <20%. The LOQ is found to be 2.0 ng/ml

2. **Specificity / Cross Reactivity:**
Specificity of an analytical method is defined as its ability to measure an analyte accurately in the presence of interference. Antibodies used in the kit are affinity purified. We have found that antibodies used in this kit are very specific.
The Endonuclease Serratia marcescens used to generate the antibodies was greater than 98% as per the SDS-Page (12%) run below.



3. 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 (5.0 ng/ml), medium (10.0 ng/ml) and high (20.0 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	<15%	<15%
Medium	<12%	<12%
High	<8%	<8%

Notes:
The emphasis of this validation has been to offer different aspects to enable user to fit the same into their overall quality control program. An overall approach to total quality has been detailed elsewhere (Juran & Gryna, 1988). Further, the quality control program needs to fit into a larger context which is dependent on the organization in which it is being conducted. There are many factors at work such as the influence of external requirements including GLP's and project related needs, the organizational policy on quality control, and other internal needs. A strong quality control program will include aspects which address these needs.

To remove sources of error and increase the reliability, greater automation can be applied to immunoassays. In the clinical application of immunoassay these sources of error have now been identified (Holzel, 1991) and the use of automation to control many of the variables is being routinely employed.

THANK YOU FOR USING KRISHGEN PRODUCT!