KRISHGEN BioSystems

VALIDATION DATA OF GENLISA™ Human Terminal Complement Complex C5b-9, TCC C5b-9 ELISA (Cat No#KBH0321)

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#2.1	**GENLISA™ Human Terminal Complement Complex C5b-9, TCC C5b-9 ELISA (Catalogue No. KBH0321)**	1st January 2023

Approved: Quality Control	Approved: Product Development
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Introduction

C5b-C9, termed the complement membrane attack complex is responsible for the cytolytic function of complement, a potent mechanism contributing to innate defence against pathogens. The C5b-9 complex has been linked to cellular signaling. The membrane-associated, pore-forming protein complex provides a mechanism for transmembrane ion fluxes. Furthermore, the nonlytic membrane-associated complex, C5b-7, can impart a signal to the target cell in the absence of pore formation or permanent cell injury.

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

1. **Sensitivity**
2. **Specificity**
3. **Precision**
4. **Recovery**
5. **Linearity**
6. **Kit Stability**

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 Quantification: 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 7.5ng/ml

2. Specificity:

The antibodies used in the kit for capture and detection are specific for TCC C5b-9 (Terminal Complement Complex C5b-9). No cross-reactivity with other analogues is observed.

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=20 assays) and inter assay (n=20 assays) reproducibility on two pools with low (12.5 ng/ml), medium (100 ng/ml) and high (800 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.

Item	Intra-Assay Precision			Inter-Assay Precision		
Sample No	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	24.8	101.8	404	24.8	104	386
Standard deviation	1.42	4.31	23.11	1.59	5.15	24.78
CV (%)	5.72	4.23	5.72	6.43	4.95	6.42

4. Recovery

Human sera was measured with two replicates and two runs (n = 5). Samples were measured using one lot of reagent. All data met our acceptance criteria for % CV and 95% (CI) Confidence Intervals for % CV.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	86 - 99	93
EDTA Plasma(n=5)	87 - 102	96
Heparin Plasma(n=5)	88 - 104	97

5. Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Human TCC C5b-9 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

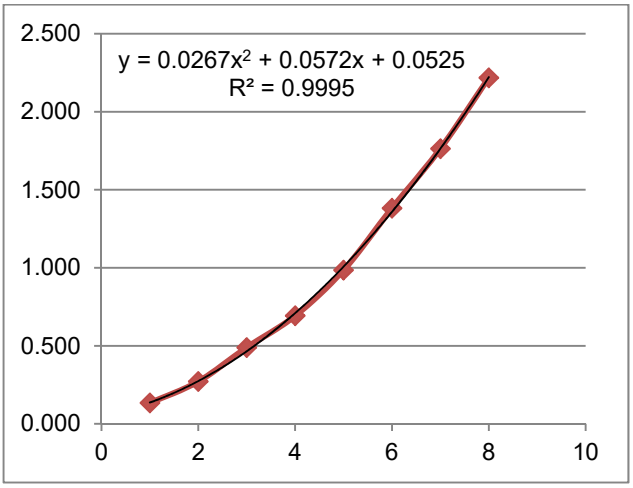
Sample	1:2	1:4	1:8
Serum(n=5)	88 - 103%	82 - 99%	82 - 97%
EDTA Plasma(n=5)	85 - 101%	84 - 100%	86 - 101%
Heparin Plasma(n=5)	87 - 105%	90 - 99%	92 - 102%

6. Kit Stability

Shelf-Life Stability: An accelerated stability study set the shelf-life stability of GENLISA™ Human Terminal Complement Complex C5b-9, TCC C5b-9 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.

7. Kit Lot Used For Validation and Data Reduction Details:

Standard Concentration (ng/ml)	Abs A	Abs B	Mean Abs
0	0.135	0.132	0.134
12.5	0.268	0.275	0.272
25	0.477	0.501	0.489
50	0.677	0.708	0.693
100	0.962	1.009	0.986
200	1.351	1.413	1.382
400	1.736	1.792	1.764
800	2.214	2.222	2.218



Conclusion:
Data reduction is done utilizing 4PL (2nd order) regression.
The kit is functional and validated for release.

QA Executive