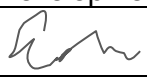
VALIDATION OF KRIBIOLISA™ Anti-Tenecteplase (TNKASE/METALYSE) ELISA KIT (Catalog No. KBI9062) AS PER FDA GUIDELINES FOR BIOANALYTICAL METHOD VALIDATION

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#1	**KRIBIOLISA™ Anti-Tenecteplase (TNKASE/METALYSE) ELISA KIT (Catalog No. KBI9062)**	18th December 2022

Approved Quality Control	Approved Product Development
	
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Introduction

This document presents a discussion of the characteristics of our **KRIBIOLISA™ Anti-Tenecteplase (TNKASE/METALYSE) ELISA (Catalogue no. KBI9062).** 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. The document is prepared based on tests run in our laboratory and does not necessarily seek to cover the testing that may be required at user's end for registration in, or regulatory submissions. The objective of this validation is to demonstrate that it is suitable for its intended purpose - detection of antibodies to Tenecteplase**.**

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

- **Limit Of Detection**
- **Specificity**
- **Precision**
- **Traceability and Stability**
- **Matrix Effect / Recovery**
- **Validation kit lot specific details**
- **Lot-to-Lot Consistency**

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.

Background

Tenecteplase is a fibrin-specific tissue-plasminogen activator. It binds to fibrin rich clots and cleaves the Arg/Val bond in plasminogen to form plasmin. Plasmin in turn degrades the fibrin matrix of the thrombus, thereby exerting its thrombolytic action. This helps eliminate blood clots or arterial blockages that cause myocardial infarction. Tenecteplase binds to fibrin rich clots via the fibronectin finger-like domain and the Kringle 2 domain. The protease domain then cleaves the Arg/Val bond in plasminogen to form plasmin. Plasmin in turn degrades the fibrin matrix of the thrombus, thereby exerting its thrombolytic action.

FDA has approved Tenecteplase as one of the protein-based therapies classified under thrombolytic agents. Commercially it is available as a drug under the trade name TNKase™ from Roche and Metalyse™ from Boehringer Ingelheim.

Validation Parameters:

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 110 ng/ml. Each lot is optimized to ensure high signal, low background, and the consistent sensitivity.

2. Specificity:

The coat protein is a tissue plasminogen activator produced by recombinant DNA technology using an established mammalian cell line to capture the specific antibodies. The standards used in the kit are polyclonal antibodies raised against recombinant tPA (tPA fusion protein Ag0200).

3. Precision:

Inter/Intra Assay 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 (125 ng/ml), medium (1000 ng/ml) and high (8000 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%	<15%
Medium	<12%	<12%
High	<10%	<10%

4. Traceability and Stability:

4.1 Traceability:

The standards used in this kit have been calibrated against commercially sourced recombinant anti-Tenecteplase antibodies.

4.2 Stability:

Shelf-Life Stability: An accelerated stability study set the shelf-life stability of KRIBIOLISA™ Anti-Tenecteplase (TNKASE/METALYSE) ELISA was performed, and a shelf life of 12 months was assigned to the kit.

5. Serum/Plasma Matrix Studies / Recovery:

Normal human serum was used to spike at (8000 ng/ml) (being the highest standard) to check the recovery at different dilutions to observe optimal recoveries. Serum spiked at (8000 ng/ml) with Anti-Tenecteplase standard and dilution done for analysis at 1:10, 1:100 and 1:1000 of serum and plasma respectively. It was seen observed 1:1000 dilution reported the most optimal recoveries.

Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:1000 Human Serum	0	0.238
	8000	2.107

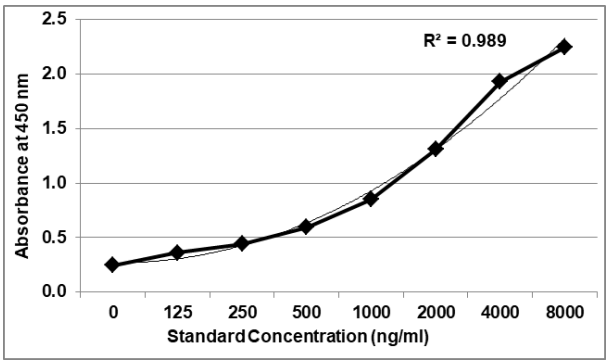
Standard Diluent	Standard (ng/ml)	Mean Abs @ 450nm
1:1000 Human Plasma	0	0.348
	8000	2.129

6. Validation Kit Specific Details:

Typical Data

Standard Concentration (ng/ml)	Mean Absorbance	Interpolated Concentration	% Interpolated Concentration against Actual Concentration
0	0.245	--	--
125	0.362	142.1	113.7
250	0.442	295.7	118.3
500	0.596	537.7	107.5
1000	0.851	953.9	95.4
2000	1.310	1902.1	95.1
4000	1.929	4370.8	109.3
8000	2.244	7563.0	94.5

Typical Graph



7. Lot-to-Lot Consistency

All lots are tested to ensure low background, a linear standard curve, consistent assay sensitivity, and a broad dynamic standard curve range. Consistent standard curve O.D.s and control values ensure that sample data is comparable over time.