KRISHGEN BioSystems
OUR REAGENTS, YOUR RESEARCH

VALIDATION OF Human N-methyl-d-aspartic Acid, NMDA GENLISA™ ELISA (CATALOG NO. KBH1051) 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#1.0	**Human N-methyl-d-aspartic Acid, NMDA GENLISA™ ELISA (CATALOG NO. KBH1051)**	01.08.2023

Approved Quality Control	Approved Product Development
Ankita G	Atul G

Introduction

This document presents a discussion of the characteristics of our **Human N-methyl-d-aspartic Acid, NMDA GENLISA™ ELISA (CATALOG NO.KBH1051)** kit considered by us during the validation of this kit in accordance with ICH Q2 (R1) guidelines. 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 **N-methyl-d-aspartic Acid, NMDA.**

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

- **Sensitivity**
- **Specificity / Cross reactivity**
- **Precision**
- **Linearity**
- **Recovery Rate**

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

N-methyl-D-aspartic acid or N-methyl-D-aspartate is an amino acid derivative that acts as a specific agonist at the NMDA receptor mimicking the action of glutamate, the neurotransmitter which normally acts at that receptor. Unlike glutamate, NMDA only binds to and regulates the NMDA receptor and has no effect on other glutamate receptors (such as those for AMPA and kainate). NMDA receptors are particularly important when they become overactive during, for example, withdrawal from alcohol as this causes symptoms such as agitation and, sometimes, epileptiform seizures.

1. Sensitivity:

Limit Of Quantification:
It is defined as the lowest detectable concentration that can be determined with an acceptable repeatability and the LOQ was found to be 4.25ng/ml.

2. Specificity / Cross Reactivity:

Specificity:

The antibodies used in this kit are monoclonal antibodies specific for Human N-methyl-d-aspartic Acid, NMDA. No Cross-reactivity with other analogues was observed.

3. Precision:

3.1 Inter/Intra Assay Precision:

Three samples of known concentration were tested twenty times on one plate to assess.
Intra-assay Precision (Precision within an assay): CV% <15%

Three samples of known concentration were tested in twenty assays to assess.
Inter-assay Precision (Precision between assays): CV% <15%

4. Linearity

To assess the linearity of the assay, samples (n=4) were spiked with high concentrations of NMDA in various matrices and diluted with the Sample Diluent to produce samples with values within the dynamic range of the assay.

Dilution	Average %	Range %
1:1	93	90-97
1:2	95	91-99
1:4	95	88-101
1:8	95	90-100

5. Recovery rate

The recovery of NMDA spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay as directed in the Sample Preparation section.

Sample Type	Recovery Average %	Range
Serum (n=5)	95	94-101
EDTA plasma (n=4)	96	92-103