Certificate of Analysis

Product Name: ELISA Kit for Nicotinamide Adenine Dinucleotide (NAD)

Cat.No.: AAA22856 Organism: Pan-species (General)

Lot.No. L240610299

Introduction

Item	Standard		Test Result
Description	This immunoassay kit allows for the specific measurement of NAD concentration in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.		Conform
Identification	Colorimetric		Positive
Composition	Pre-coated, ready to use 96-well strip plate Standard Standard Diluent Detection Reagent A (green) Detection Reagent B (red) Assay Diluent A Assay Diluent B TMB Substrate Stop Solution Wash Buffer(30 x concentrate) Plate sealer for 96 wells Instruction manual	1 2 1 × 20ml 1 × 120µl 1 × 120µl 1 × 12ml 1 × 12ml 1 × 9ml 1 × 6ml 1 × 20ml 4 1	Conform
Assay Range	123.5-10,000ng/mL		Conform

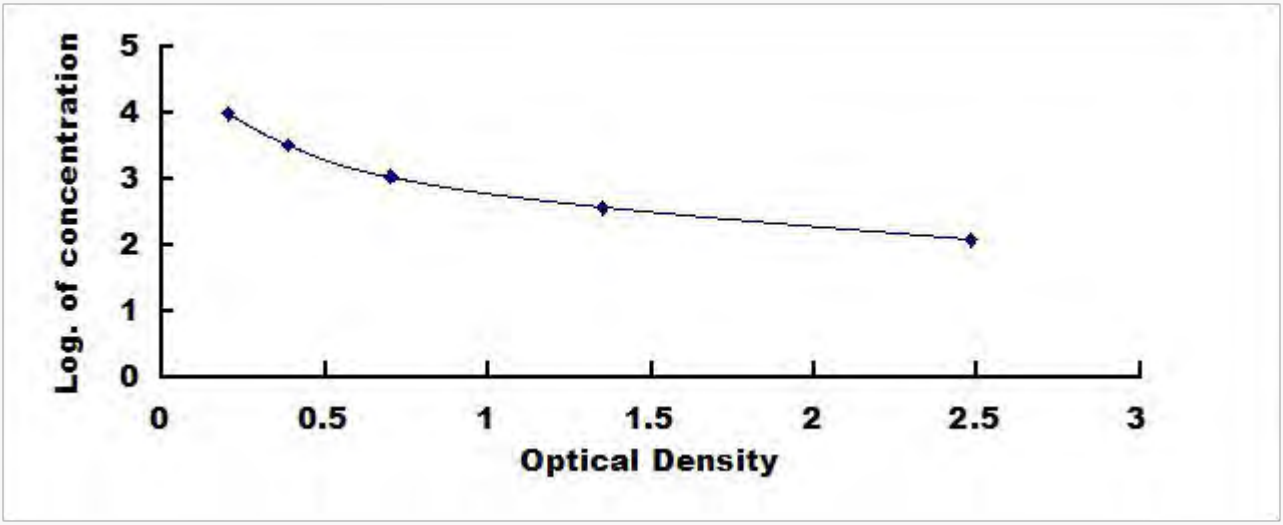
Sensitivity

The minimum detectable dose of NAD is typically less than 49.3ng/mL.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D. Value of 20 replicates of the zero standard added by their three standard deviations.

Standard curve

The standard curve is provided for demonstrated only. The client should perform the standard test in each independent experiment.

ng/ml	Standard		Average	Log of concentration
123.5	2.496	2.471	2.484	2.092
370.4	1.371	1.337	1.354	2.569
1111.1	0.698	0.691	0.695	3.046
3333.3	0.392	0.392	0.392	3.523
10000	0.208	0.204	0.206	4.000



Recovery

Different matrices were spiked with certain level of NAD and the recovery rates were evaluated.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	83-96	90
EDTA plasma(n=5)	90-105	99
heparin plasma(n=5)	81-95	87

Linearity

The linearity of the kit was assayed by testing samples spiked with high concentration of NAD and also several dilutions of these samples. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1：2	1：4	1：8	1：16
serum(n=5)	83-95%	85-99%	92-103%	79-89%
EDTA plasma(n=5)	81-96%	78-91%	90-106%	82-98%
heparin plasma(n=5)	87-102%	84-97%	89-101%	80-94%

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level of NAD were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of NAD were tested on 3 different plates, 8 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/ml)	371.27	1112.98	3333.25	380.36	1113.94	3335.32
SD	15.642	59.244	221.894	17.287	63.450	225.101
CV (%)	4.2	5.3	6.7	4.5	5.7	6.7

Date: *2024.06.17*