

## Canine NT-ProBNP ELISA Kit

**Catalog Number: orb441263**

### Certificate of analysis

#### Introduction

Item	Standard	Test Result																								
Description	The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of NT-ProBNP in canine serum, plasma, tissue homogenates, cell lysates, cell culture Supernates or biological fluids.	Conform																								
Identification	Colorimetric	Positive																								
Composition	<table border="1"> <tbody> <tr> <td>Pre-coated, ready to use 96-well strip plate</td> <td>1</td> </tr> <tr> <td>Standard (freeze dried)</td> <td>2</td> </tr> <tr> <td>Standard Diluent</td> <td>1 × 20ml</td> </tr> <tr> <td>Detection Reagent A</td> <td>1× 120µl</td> </tr> <tr> <td>Detection Reagent B</td> <td>1× 120µl</td> </tr> <tr> <td>Assay Diluent A</td> <td>1 × 12ml</td> </tr> <tr> <td>Assay Diluent B</td> <td>1 × 12ml</td> </tr> <tr> <td>TMB Substrate</td> <td>1 × 9ml</td> </tr> <tr> <td>Stop Solution</td> <td>1 ×6ml</td> </tr> <tr> <td>Wash Buffer(30 x concentrate)</td> <td>1 ×20ml</td> </tr> <tr> <td>Plate sealer for 96 wells</td> <td>2</td> </tr> <tr> <td>Instruction manual</td> <td>1</td> </tr> </tbody> </table>	Pre-coated, ready to use 96-well strip plate	1	Standard (freeze dried)	2	Standard Diluent	1 × 20ml	Detection Reagent A	1× 120µl	Detection Reagent B	1× 120µl	Assay Diluent A	1 × 12ml	Assay Diluent B	1 × 12ml	TMB Substrate	1 × 9ml	Stop Solution	1 ×6ml	Wash Buffer(30 x concentrate)	1 ×20ml	Plate sealer for 96 wells	2	Instruction manual	1	Conform
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Assay Range	78.125-5000pg/mL	Conform																								

#### Sensitivity

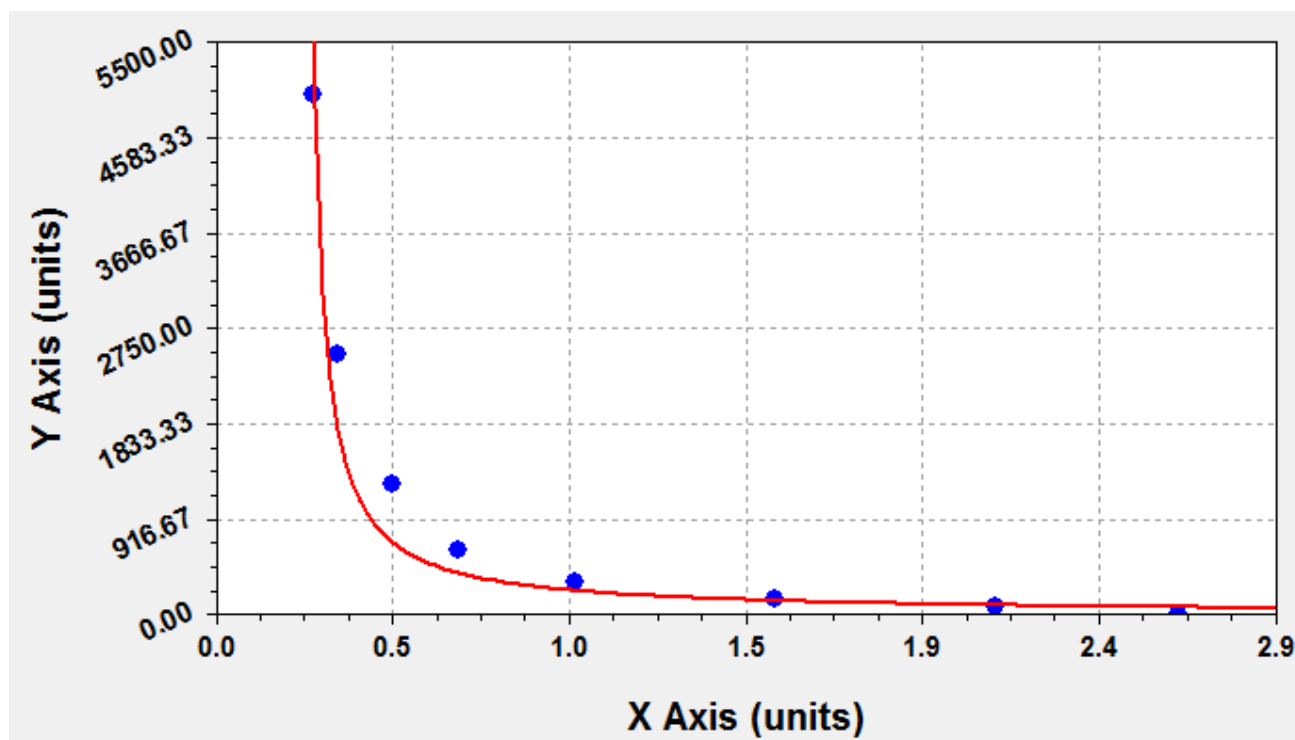
The minimum detectable dose of canine NT-ProBNP is typically less than 28pg/mL.

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 by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

#### Standard curve

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

pg/mL	Standard		Average	Corrected
0	2.646	2.654	2.65	---
78.1	2.141	2.151	2.146	---
156	1.533	1.543	1.538	---
312	0.982	0.992	0.987	---
625	0.656	0.674	0.665	---
1250	0.499	0.471	0.485	---
2500	0.321	0.343	0.332	---
5000	0.264	0.272	0.268	---



### Recovery

Matrices listed below were spiked with certain level of recombinant canine NT-ProBNP and the recovery rates were calculated by comparing the measured value to the expected amount of NT-ProBNP in samples.

Matrix	Recovery range (%)	Average (%)
serum(n=5)	87-90%	88%
EDTA plasma(n=5)	83-103%	93%
heparin plasma(n=5)	81-86%	84%

## Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of canine NT-ProBNP and their serial dilutions. 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)	92-101%	91-100%	86-96%	82-92%
EDTA plasma(n=5)	92-101%	87-100%	90-98%	82-99%
heparin plasma(n=5)	83-102%	79-98%	87-96%	91-101%

## Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level canine NT-ProBNP were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level canine NT-ProBNP were tested on 3 different plates, 8 replicates in each plate.

$$CV(\%) = SD/\text{mean} \times 100$$

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	8	8	8
Mean (pg/mL)	915.07	2754.90	4845.43	548.770	2542.94	3617.00
SD	66.98	186.78	392.48	33.31	203.69	263.32
CV (%)	7.32	6.78	8.10	6.07	8.01	7.28

Approved Date: Jan.14,2018

Q.C. Manager Signed: Chen