

## Certificate of analysis

**Product Name:** ELISA Kit for Melatonin (MT)

**Cat.No.:** AAA22832 **Organism:** Pan-species (General)

**Lot.No.** L240227023

### Introduction

Item	Standard		Test Result
Description	This immunoassay kit allows for the specific measurement of melatonin concentration in serum, plasma and other biological fluids.		Conform
Identification	Colorimetric		Positive
Composition	Pre-coated, ready to use 96-well strip plate	1	Conform
	Standard	2	
	Standard Diluent	1 × 20ml	
	Detection Reagent A (green)	1 × 120μl	
	Detection Reagent B (red)	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	4	
	Instruction manual	1	
Assay Range	12.35-1,000pg/mL		Conform

### Sensitivity

The minimum detectable dose of melatonin is typically less than 4.53pg/mL.

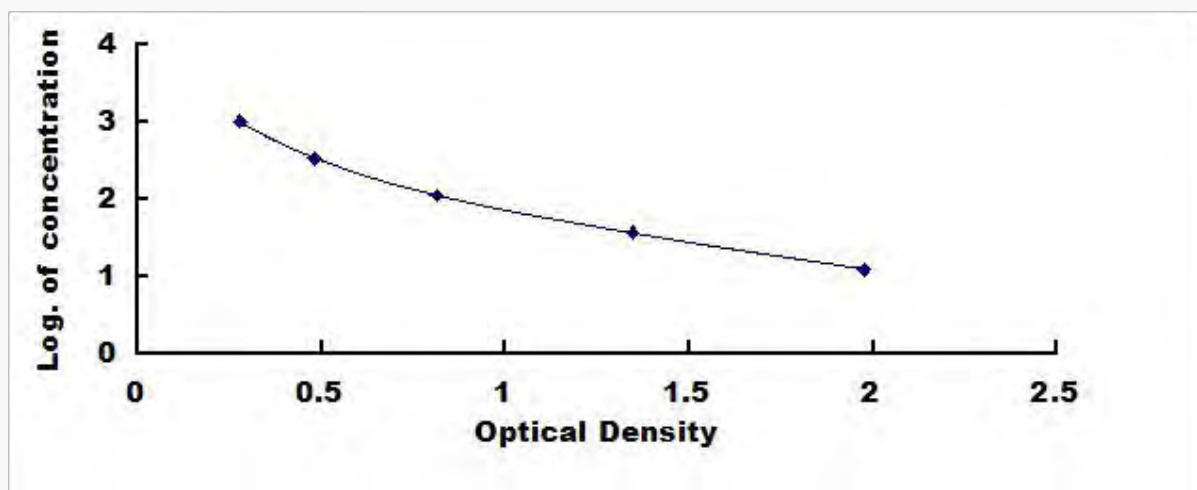
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined the mean O.D. Value of 20 replicates of the zero standard added by their three standard deviations.

### Standard curve

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



pg/ml	Standard		Average	Log of concentration
12.35	1.998	1.945	1.972	1.092
37.04	1.374	1.312	1.343	1.569
111.11	0.798	0.823	0.811	2.046
333.33	0.467	0.479	0.473	2.523
1000	0.269	0.274	0.272	3.000



## Recovery

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

Matrix	Recovery range (%)	Average(%)
serum(n=5)	84-94	90
EDTA plasma(n=5)	81-95	88
heparin plasma(n=5)	89-101	95

## Linearity

The linearity of the kit was assayed by testing samples spiked with high concentration of melatonin 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)	86-101%	84-98%	83-97%	90-107%
EDTA plasma(n=5)	78-90%	95-109%	80-93%	91-105%
heparin plasma(n=5)	87-104%	81-93%	79-99%	88-101%

**Precision**

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

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level of melatonin 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 (pg/ml)	36.27	115.98	336.34	38.78	118.24	342.69
SD	1.542	6.534	22.689	1.797	6.794	23.608
CV (%)	4.3	5.6	6.7	4.6	5.7	6.9

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