Human Melatonin ELISA Kit

Enzyme Immunoassay for the quantification of human Melatonin in human saliva.

Catalog number: ARG82780

TABLE OF CONTENTS

SECTION **Page**

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Melatonin is a hormone primarily released by the pineal gland at night, and has long been associated with control of the sleep–wake cycle. As a dietary supplement, it is often used for the short-term treatment of insomnia, such as from jet lag or shift work, and is typically taken by mouth.

In vertebrates, melatonin is involved in synchronizing circadian rhythms, including sleep–wake timing and blood pressure regulation, and in control of seasonal rhythmicity including reproduction, fattening, moulting and hibernation. Many of its effects are through activation of the melatonin receptors, while others are due to its role as an antioxidant. In plants, it functions to defend against oxidative stress. It is also present in various foods.

In humans, melatonin is a full agonist of melatonin receptor 1 (picomolar binding affinity) and melatonin receptor 2 (nanomolar binding affinity), both of which belong to the class of G-protein coupled receptors (GPCRs). Melatonin receptors 1 and 2 are both Gi/o-coupled GPCRs, although melatonin receptor 1 is also Gq-coupled. Melatonin also acts as a high-capacity free radical scavenger within mitochondria which also promotes the expression of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase via signal transduction through melatonin receptors

Melatonin is metabolized in the liver by cytochrome P450 enzyme CYP1A2 to 6-hydroxymelatonin. Metabolites are conjugated with sulfuric acid or glucuronic acid for excretion in the urine. 5% of melatonin is excreted in the urine as the unchanged drug.

The membrane transport proteins that move melatonin across a membrane include, but are not limited to, glucose transporters, including GLUT1, and the proton-driven oligopeptide transporters PEPT1 and PEPT2.

For research as well as clinical purposes, melatonin concentration in humans can be measured either from the saliva or blood plasma. [Provide from Wikipedia: Melatonin]

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. Endogenous Melatonin of a patient sample competes with a biotinylated-Melatonin for binding to the fixed number of Melatonin antibody binding sites. The amount of bound biotin conjugate is inversely proportional to the concentration of Melatonin in the sample. After incubation the unbound conjugate is washed off. The antibody-bound biotinylated antigen is determined by the use of streptavidin-peroxidase as marker and TMB as substrate. The reaction is monitored by a color change which is readable at OD of 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards. The intensity of color development is inversely proportional to the amount of Melatonin in the samples.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 plate (8 X 12 strips)	4°C
Standard 0-5 (0.0, 0.5, 1.5, 5.0, 15.0, 50.0 pg/ml)	6 x 1 ml (Ready to Use)	4°C
Control 1	1 X 1 ml (Ready to Use)	4°C
Control 2	1 x 1 ml (Ready to Use)	4°C
Melatonin Antibody	1 X 7 ml (Ready to Use)	4°C
Melatonin Biotin	1 X 12 ml (Ready to Use)	4°C
Streptavidin-HRP conjugate	1 X 12 ml (Ready to Use)	4°C
20X Wash buffer	1X 50 ml	4°C
TMB substrate	1 X 15 ml (Ready to Use)	4°C (Protect from light)
STOP solution	1 X 15 ml (Ready to Use)	4°C
Adhesive foil	3	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm (reference wavelength 600-650 nm)
- Pipettes and pipette tips
- Deionized or distilled water
- Orbital microplate shaker (400 – 600 rpm)
- Centrifuge (preferably refrigerated) 2000- 3000 x g

- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. Keep away from heat or direct sunlight.
- The microtiter strips are stable up to the indicated expiry after the kit is opened. Make sure that the opened bag is tightly closed when stored at 2- 8 °C.
- Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.
- Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18 °C- 25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming. Briefly spin down the vials before use.

- Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system.
- Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Incubation time affects results. All wells should be handled in the same order and time sequences.
- The relative centrifugal force (g) is not equivalent to rounds per minute (rpm) but it has to be calculated depending on the radius of the centrifuge.
- It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Notice:

- The patient should not eat, drink, chew gums or brush teeth for 30 min before sampling.
- Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).
- Reddish color is indicating blood contamination and leading to wrong results.
- Samples should not be taken from patients that took biotin-containing multivitamins or supplements within last 48 h.

Collection:

- Rinse mouth thoroughly with cold water 5 min prior to sample collection. Saliva flow can be stimulated by chewing on a piece of Parafilm
- Saliva can be collected in a suitable sampling device. Sample collection systems which contain cellulose pads should not be used.
- A minimum of 0.5 ml liquid should be collected. It is recommended to freeze samples at –20 °C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.

Storage:

- Saliva samples can be stored at room temperature for 1 day or at 2 – 8 °C for 7 days.
- Aliquot and store samples at -20°C is recommended. Sample can be stored at-20 °C for up to 6 months. Avoid repeated freeze-thaw cycles. Keep away from heat or direct sunlight.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into **distilled water** to yield 1X Wash buffer. E.g.: Add 10 ml of 20X Wash buffer into 190 ml of distilled water to a final volume of 200 ml, mix thoroughly. The diluted Wash buffer is stable for 4 weeks at 2-8°C.
- Samples: Samples suspected to contain concentrations higher than the highest standard have to be diluted with **Standard 0** prior to extraction step. Dilution has to be made in glass tubes. Measured results have to be multiplied by dilution factor to obtain corrected results. Values lower than 0 pg/ml should be repeated by an additional measurement

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT; 18- 25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of standards, controls and samples in duplicate into the respective wells of the Microtiter Plate.
3. Add **50 µl** of **Melatonin Antibody** into each well. Cover with adhesive foil. **Incubate for 16-20 hours at 2-8°C**.
4. Remove adhesive foil. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes**. Wash by filling each well with **1× Wash Buffer (250 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add **100 µl** of **Melatonin Biotin** into each well.
6. Cover with adhesive foil. Incubate **120 min at RT** on an orbital microplate shaker (500 rpm).
7. **Wash** as according to **step 4.**
8. Add **100 µl** of **Streptavidin-HRP conjugate** into each well. Cover plate with adhesive foil. Incubate **60 min at RT** on an orbital microplate shaker (500 rpm).
9. **Wash** as according to **step 4.**
10. Add **100 µl** of **TMB substrate** to each well. Incubate for **15 minutes at RT** on an orbital microplate shaker (500 rpm) **in dark.**
11. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
12. **Read** the OD with a microplate reader at **450 nm** (Optional: Read at 600-650 nm as reference) immediately or within 15 min after pipetting of the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear, semi-log or log-log graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

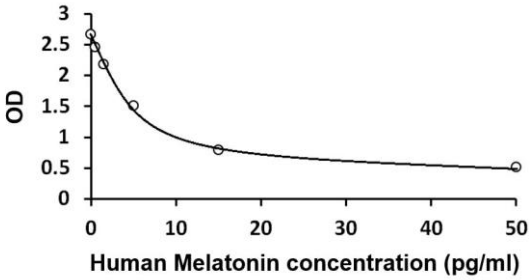
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for detail. (https://www.arigobio.com/elisa-analysis)

6. Conversion: Melatonin (pg/ml) X 4.30 = pmol/L.

7. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



EXPECTED VALUES

A study with apparently healthy subjects has shown that the melatonin levels in humans have a marked circadian rhythmicity characterized by very low levels during day-time (0- 8 pg/ml) and high levels during night-time (10- 58 pg/ml) and show a considerable inter-individual variation. The nocturnal melatonin peak among healthy individuals varies significantly. It is recommended that each laboratory establishes its own range of normal values.

Note:

The results themselves should not be the only reason for any therapeutical consequences. They have to be correlated to other clinical observations and diagnostic tests.

QUALITY ASSURANCE

Specificity

Substance	Cross reactivity
Serotonin	0.54 %
5-Methoxytryptamine	< 0.01
N-Acetylserotonin	< 0.01
5-Methoxytryptophol	< 0.01

Sensitivity

Limit of Blank (LoB): The LoB study was performed with the zero calibrator (Standard 0), measured in 28 replicates in one run. Limit of Blank = 0.4 pg/ml.

Limit of Quantitation (LoQ): The LoQ study was performed with 10 saliva samples, measured in 10 replicates in one run. Limit of Quantitation = 0.854 pg/ml (CV = 20 %)

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 13.2-19 % and inter-assay precision was 16.5-23.8%.

Recovery

74-114%