

ab283259 – Melatonin ELISA Kit

For the direct quantitative measurement of Melatonin in human serum and plasma.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab283259>

Storage and Stability

The entire ELISA kit may be stored at 4°C for up to 12 months from the date of shipment.

Opened kits retain activity for 28 days if stored as described above.

Materials Supplied

Item	Quantity	Storage Condition
Antibody Coated Wells 96 wells plate	1 x 1 unit	4°C
Melatonin Standards 1-6	6 x 500 µl	4°C
Control 1	1 x 500 µl	4°C
Control 2	1 x 500 µl	4°C
Anti-Melatonin Solution	1 x 6 ml	4°C
Enzyme Conjugate	1 x 6 ml	4°C
Wash Solution Concentrated (10X)	1 x 50 ml	4°C
TMB Reagent	1 x 15 ml	4°C
Stop Solution	1 x 15 ml	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Device to dispense accurately 25 µL and 50 µL.
- Multichannel pipettors.
- Microplate or orbital shaker
- Vortex Mixer
- Microplate washer (not required, plates can be washed manually).
- Microplate reader capable of reading 450 nm with 4 parameter data reduction or comparable software.
- Plate Sealers
- Suitable serum or plasma sample collection device.

Reagent Preparation

Wash Solution Concentrated (10X): Prior to use dilute 1:10 with deionized water.

- All other reagents are supplied ready to use.

Sample Collection and Processing

- The usual precautions for venipuncture should be observed.
- It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it assayed.
- Do not use grossly hemolytic, icteric, or grossly lipemic specimens.
- Samples appearing turbid should be centrifuged before testing to remove any particulate material.
- Samples containing Azide or thimerosal are unsuitable for this assay.

Sample Stability:

Storage	2 – 8 °C	≤-20°C (Aliquots)	≤-40°C (Aliquots)
Stability	24 hours	3 months	1 year

Keep away from heat or direct sunlight. Avoid repeated freeze-thaw cycles.

Assay Procedure

- Assay type is competitive ELISA.
- It is recommended that the calibrators, controls, and samples should be tested in duplicate and the mean value should be used to report the results.
 1. To the microplate dispense 25 µL of ready to use Melatonin Standards (0, 3, 10, 30, 100 and 300 pg/mL), controls, and samples.
 2. Add 50 µL of Enzyme Conjugate to all wells.
 3. Add 50 µL of Anti-Melatonin Solution.
 4. Cover microplate with plastic sealer. Incubate by shaking on a microplate orbital shaker set a 500 - 900 rpm for 2 hours at room temperature.
 5. After incubation, decant the contents of the wells. Wash 3 times with 300 µL of diluted Wash Solution. After the 3rd wash, invert the microplate on absorbent paper and tap dry.
 6. Dispense 125 µL of TMB Reagent into each well. Shake briefly (manual). Cover microplate with plastic sealer. Incubate for 30 minutes at room temperature.
 7. Dispense 125 µL of Stop Solution into each microtiter well of the plate. Shake briefly (manual). Color changes from blue to yellow.
 8. Read at 450 nm on a microplate reader within 10 minutes.

Δ Note: If samples exceed the upper end of the measuring range of 300 pg/mL, dilute with zero calibrator and make appropriate concentration correction.

9. Determine the concentrations of the controls and unknowns by interpolation using Software capable of logistics using a 4-parameter sigmoid minus curve fit.

Δ Note: The expected values for the controls are stated on the control labels. Results can only be accepted if expected values are met.

Expected Values

Samples from apparently healthy subjects collected show the following results below:

Time	Subjects (number)	Mean Melatonin in Serum	90 % percentile
03:00 AM	129	78.2 pg/ml	18.5-180 pg/ml
08:00 AM	128	28.5 pg/ml	3.8-80.4 pg/ml

It is recommended that each laboratory establishes its own range of normal values.

Specificity of the Antiserum

Compounds	% Cross-reactivity
N-Acetylserotonin	0.38
5-MethoxyTryptophol	<0.001
5-Methoxy-DL-Thyptophan	<0.001
Serotonin Hydrochloride	<0.001
5-Methoxytryptamine	0.15
6-Hydroxy melatonin	<0.001

Recovery

Three serum samples with different levels of endogenous Melatonin were spiked with known quantities of Melatonin.

Sample	Endogenous (pg/mL)	Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
1	5.4	50.0	55.4	58.5	105.6
2	6.9	100.0	106.9	121.4	113.6
3	4.3	200.0	204.3	208.6	102.1

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus