ab283258 – Melatonin ELISA Kit (salivary)

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

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283258

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 unit	4°C
Concentrated Stock Melatonin Calibrator	1 x 200 µl	4°C
Assay diluent	1 x 20 ml	4°C
Concentrated Stock Melatonin Control	1 x 200 µl	4°C
Anti-Melatonin Solution	1 x 3 ml	4°C
Enzyme Conjugate	1 x 700 µl	4°C
Conjugate Buffer	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 saliva sample collection device.

Reagent Preparation

Concentrated Stock Melatonin Calibrator: Dilute the 6400 pg/mL stock solution 1:100 (1 part 6400 pg/mL+ 99 parts assay diluent) to obtain the highest working calibrator (64 pg/mL) then, dilute serially 1:2 (starting with the 64 pg/mL calibrator) to obtain the following concentrations of working calibrators: 32 pg/mL, 16 pg/mL, 8 pg/mL, 4 pg/mL, 2 pg/mL and 1 pg/ml. "0" calibrator is assay diluent.

Concentrated Stock Melatonin Control:

Working Control # 2 Preparation (Example)

Concentrated Stock Melatonin Control	Assay Diluent	Dilution	Target	Number of EIA wells per 5 mL volume
0.05 mL (50 µL)	4.950 mL	1:100	30 pg/ml	100

Working Control # 1 Preparation

Working Control #2	Assay Diluent	Dilution	Target	Number of EIA wells per 5 mL volume
0.5 ml	4.5 ml	1:10	3 pg/ml	100

Immediately after use, store the unused portions of the working calibrators and the High and Low Controls at 2-8°C. Discard if not used within 7 days of mixing.

Conjugate Buffer: Use only for the preparation of the Melatonin-HRP working reagent.
Melatonin-HRP working reagent preparation: Determine the amount of working Melatonin HRP needed and dilute 1:10 with conjugate buffer pH 7.4. For example, mix 0.5 mL of Enzyme Conjugate plus 4.5 mL with conjugate buffer. This is sufficient for 100 EIA wells. The Melatonin HRP working reagent is light sensitive. Immediately after use, wrap the vial with the unused portion of the Melatonin-HRP working reagent with aluminum foil or alternatively, prepare the Melatonin-HRP working reagent in an amber vial. Store at 2-8°C. Discard if not used within 7 days of mixing.

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

Sample Collection:

1. Rinse mouth thoroughly with cold water 5 minutes prior to sample collection.
2. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).
3. Saliva can be collected in a suitable sampling device. A minimum of 0.5 ml liquid should be collected.
4. After collection, refrigerate sample within 30 minutes and freeze at or below -20°C within 4 hours of collection. On day of assay thaw the saliva samples, vortex, and centrifuge at 1500x for 15 minutes. Dispense clear sample into appropriate wells.
5. Samples containing Azide or thimerosal are unsuitable for this assay.

Sample Stability:

Storage	Room Temperature 20 – 30 °C	37 °C	2 – 8 °C	≤ -15 °C (freeze / thaw
Stability	Up to 7 days	Up to 7 days	Up to 7 days	Up to 7 times

Assay Procedure

- 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 microtitre plate dispense 50 µL of working Melatonin calibrators (0, 1, 2, 4, 8, 16, 32 and 64 pg/mL), controls, and saliva samples.
2. Add 50 µL of Melatonin-HRP Working Reagent to all wells.
3. Add 25 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 microplate on an 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 64 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

Saliva samples from apparently healthy subjects collected in the PM (bedtime), AM (arise), and noon show the following results below:

Time	Subjects (number)	Median (pg/ml)	Range (pg/ml)
pm	27	6.4	1.4 – 24.2
am	27	6.3	1.6 – 22.6
noon	27	2.7	0.2 – 10.2

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-MethoxyTrytophol	<0.001
5-Methoxy-DL-Thyptophan	<0.001
Serotonin Hydrochloride	<0.001
5-Methoxytryptamine	0.15
6-Hydroxy melatonin	<0.001

Recovery

Four saliva 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	11.0	20.0	31.0	33.4	107.7
2	8.0	10.0	18.0	17.0	94.4
3	2.1	20.0	22.1	22.9	103.6
4	17.2	10.0	27.2	29.1	107.0

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