May 22, 2024

Melatonin ELISA

DOI
dx.doi.org/10.17504/protocols.io.81wgbx35ylpk/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.81wgbx35ylpk/v1

Protocol Citation: daniel.dautan daniel, Per Svenningsson 2024. Melatonin ELISA. **protocols.io https://dx.doi.org/10.17504/protocols.io.81wgbx35ylpk/v1**

Protocol status: Working
We use this protocol and it's working

Created: February 15, 2024

Last Modified: September 23, 2024

Protocol Integer ID: 95309

Keywords: ASAPCRN, melatonin, sleep, melatonin elisa measurement of mouse plasma melatonin, melatonin elisa measurement, mouse plasma melatonin, using elisa kit, elisa kit, mouse plasma

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: 020608

Abstract

Measurement of mouse plasma melatonin using ELISA Kit (Enzo Life Sciences, ENZ-KIT150-0001, NY, US) according to manufacturer instructions.

Materials

Enzo Life Sciences, ENZ-KIT150-0001

Troubleshooting

Melatonin Extraction

1 Mix 200 µL of plasma with an equal volume of cold Ethyl Acetate. Vortex gently.

2 Allow layers to separate on ice for 00:03:00. Vortex again and incubated On ice for 00:02:00. 5m

3 Spin samples at 1000g for 00:10:00 at 4 °C. Transfer the organic layer to a new tube. 10m

4 Dry samples and resuspend in 220 µL of 1X stabilizer.

ELISA 2h

5 Add 100 µL of standards' working solutions and samples to provided 96-well plate in duplicates.

6 Immediately after add 50 µL of melatonin tracer to each well (except blanks) followed by 50 µL of 1X melatonin antibody (except blanks).

7 Cover plates with the provided plate sealer. Incubate for 01:00:00 at 37 °C with 500 rpm shaking. 1h

8 Decant the solution from each well. Add 400 µL of wash solution to each well.

9 Decant the solution from each well and pat dry against clean absorbent paper.

10 Repeat wash step 3 times.

11 Add 200 µL of melatonin conjugate solution to each well (except blanks). Cover plate with the sealer. Incubate for 00:30:00 at Room temperature. 30m

12 Perform the wash step as described above. Add 200 µL of substrate reagent to each well. Incubate for 00:30:00 at 37 °C protected from light. 30m

13 Add 50 µL of stop solution to each well. Measure the optical density using a micro-plate reader with absorbance set to 450 nm.