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## Orexin A Analysis in Plasma

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**We use this protocol and it's working**

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## Abstract

Mouse plasma analysis of Orexin A using Novus Biologicals ELISA kit (NBP2-80231).

## Materials

ELISA Orexin A kit- Novus Biologicals, NBP2-80231, MO, US



- 1 Centrifuge samples for 00:15:00 at 1000×g at 4 °C . 15m
- 2 Add 50 µL of standards' working solutions and samples to 96-well plate in duplicates.
- 3 Add 50 µL of biotinylated detection antibody working solution to each well. Cover with the provided plate sealer and incubate for 00:45:00 at 37 °C . 45m
- 4 Decant the solution from each well. Add 350 µL of wash buffer added to each well and allowed to soak for 00:01:00 . 1m
- 5 Decant the solution from each well and pat dry against clean absorbent paper. Repeat this wash step (steps 4-5) 3 times.
- 6 Add 100 µL of HRP conjugate working solution to each well. Cover plate with the sealer and incubate for 00:30:00 at 37 °C . 30m
- 7 The wash step was performed as described above. Add 90 µL of substrate reagent to each well. Incubate for 00:15:00 at 37 °C protected from light. 15m
- 8 Add 50 µL of stop solution to each well. Measure the optical density using a micro-plate reader with absorbance set to 450 nm.