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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

Troubleshooting

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.