Mouse Oxytocin ELISA Kit

Catalog #: NB-E20200 (96 wells)

User Manual

This kit is designed to quantitatively detect the levels of Mouse Oxytocin in cell lysates, serum/ plasma and other suitable sample solution.

Manufactured and Distributed by:

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Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially:

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrate, it may be appropriate to extend the incubation time (Do not over-develop).
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS.

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Scientific Background

The neuropeptides, oxytocin and vasopressin were isolated and synthesized by Vincent du Vigneaud at Cornel Medical College in 1953, work for which he received the Nobel Prize in Chemistry in 1955. Oxytocin is a neurohypophysial peptide which is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior pituitary. The molecule consists of nine amino acids linked with a [1-6] disulfide bond and a semi-flexible carboxyamidated tail. A hormone once thought to be limited to female smooth muscle reproductive physiology and neurotransmitter 1,2, recent studies have begun to investigate oxytocin's role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors 3,4 and is important in male reproductive physiology5. Oxytocin and the related neurohypophysial peptide, Arg8-Vasopressin, maintain renal water and sodium balance6.

Highly conserved across species boundaries, oxytocin-like neurohypophysial peptides are substituted primarily at residues 4 and/or 8. In the oxytocin-like peptide, mesotocin, a common peptide found in some fishes, reptiles, birds, amphibians, marsupials and non-mammalian tetrapods, the leucine at residue 8 is substituted for isoleucine 7. Acting in classical endocrine fashion, Oxytocin elicits regulatory effects by binding specific cell surface receptors which in turn initiate a secondary intracelluar response cascade via a phosphoinositide signaling pathway.

Intended use

The kit is used to quantify the Mouse Oxytocin in serum/ plasma, cell culture supernatant and other suitable sample solution.

Standard range	16.38 – 10,000 pg/mL
Assay time	4.5 hours
Validity	Six months
Store at	2-8 °C

Assay principle

The Oxytocin Immunoassay kit is designed to quantitatively measure Oxytocin present in serum, plasma, saliva, clarified milk and tissue culture media samples. Please read the complete kit insert before performing this assay. An oxytocin standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An oxytocin-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a polyclonal antibody to oxytocin to each well. After an overnight incubation at 4°C the plate is washed and supplied substrate is added. The substrate reacts with the bound oxytocin-peroxidase conjugate. After a 30 minute incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the oxytocin in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers.

Materials supplied

1. Mouse Oxytocin standard:	125 µl
2. 96-well plate pre-coated with Goat anti-rabbit igG:	1
3. Oxytocin antibody:	3 ml
4. Oxytocin Conjugate:	3 ml
5. Assay Buffer Concentrate:	28 ml
7. Extraction Solution:	50 ml
8. TMB Substrate:	11 ml
9. Plate sealer:	1
10. Stop Solution:	5 ml
11. 20 × Wash Buffer:	30 ml

Materials required but not supplied

- 1x PBS.
- Standard plate reader capable of measuring absorbance at 450 nm.
- Adjustable pipettes and disposable pipette tips.
- Multi-channel pipettes, manifold dispenser or automated microplate washer.
- Distilled water.
- Absorbent paper.
- Materials used for sample preparation.

Sample Preparation and storage

Serum and Plasma Samples Serum and plasma samples should be extracted with the provided Extraction Solution, prior to running in the kit.

Protocol Using Extraction Solution:

1. Mix 1 part sample with 1.5 parts of Extraction Solution.
2. Vortex and then nutate at room temperature for 90 minutes.
3. Centrifuge for 20 minutes at 4°C at 1660 x g.
4. Transfer supernatant to a clean tube.
5. Speedvac supernatant to dryness at 37°C.
6. Reconstitute sample with 250 µL of Assay Buffer.

Saliva Samples Saliva samples should be extracted using the extraction reagent as described for serum and plasma samples. Saliva should be collected with Sarstedt Salivettes, extracted, dried, and reconstituted in 250 µL of Assay Buffer.

Milk Samples Milk samples should be clarified by centrifuging at 10,000 x g for 15 minutes. Pierce the top fatty layer and collect the supernatant liquid. Repeat the centrifugation and collection two more times. The collected supernatant liquid must then be diluted ≥ 1:10 with the provided Assay Buffer before using in the assay. The clarified milk sample, i.e., the supernatant liquid, can be stored at -20°C until needed.

Use all samples within 2 hour of preparation

Reagent Preparation

Standard

- Mouse Oxytocin: Standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard (100 ng /ml) are included in each kit. Use one tube for each experiment.
- Prepare 10,000 – 16.38 pg/ml of Mouse Oxytocin standard solutions:
- Add 450 ul of Sample Diluent Buffer into one standard vial into 50 ul of 100 ng/ml Mouse Oxytocin. Keep the tube at room temperature for 10 minutes and mix thoroughly. This is 10000 pg/ml standard solution.

- Label 6 Eppendorf tubes with 4000 pg/ml, 1600 pg/ml, 640 pg/ml, 256 pg/ml, 102.4 pg/ml, 40.96 pg/ml and 16.38 pg/ml respectively and transfer 300 ul of sample diluent buffer in each tube. Take 200 ul of oxytocin stock solution in tube#1 and add it to tube#2. Repeat this serial dilution for tube#3 to tube#8
- Make sure each tube has ≥ 250 µl of standard.

Note: *The standard solutions are best used within 2 hours.*

Sample diluent, antibody diluent, and HRP diluent

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Preparation of TMB Substrate solution

- The solution should be prepared no more than 10 min prior to the experiment.
- The total volume should be: 0.1 ml/well x the number of wells (allowing 0.1-0.2 ml more than total volume).
- Mix equal volumes of chromogen solutions A and B. Protect from light.

Wash Buffer

- If crystals have formed in the 20X wash buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
- Dilute 25 ml Wash Buffer Concentrate (20X) to a total volume of 500 ml with distilled water.

Assay Procedure

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine oxytocin concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.

2. Pipet 100 µL of samples or standards into wells in the plate.

3. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.

4. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.

5. Add 25 µL of the Oxytocin Conjugate to each well using a repeater pipet.

6. Add 25 µL of the Oxytocin Antibody to each well, **except the NSB wells**, using a repeater pipet.

7. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and store at 4°C for 16-18 hours.

8. The following day, remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. **Addition of cold Substrate will cause depressed signal.**

9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.

10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.

11. Incubate the plate at room temperature for 30 minutes without shaking.

12. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet.

13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.

14. Use the plate reader's built-in 4PLC software capabilities to calculate oxytocin concentration for each sample.

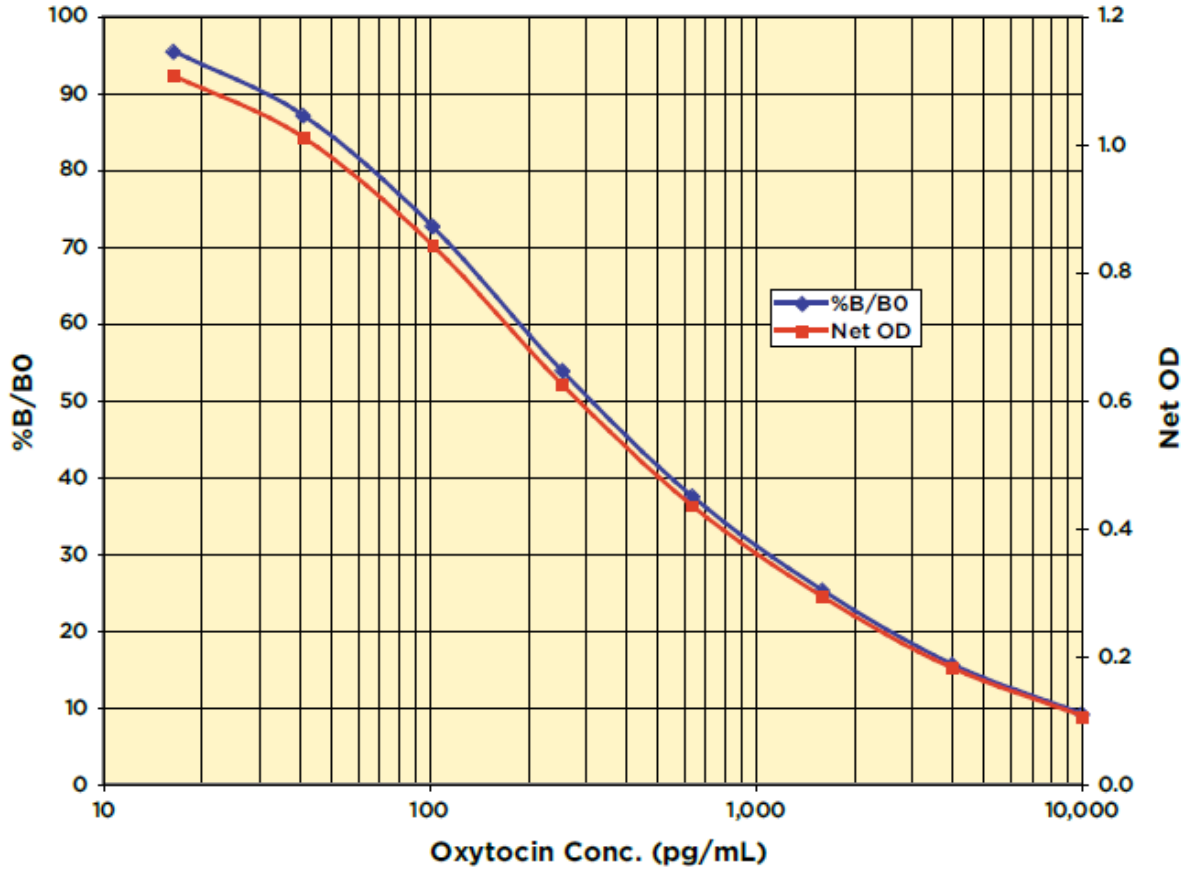
Result calculation

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Mouse Oxytocin concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Typical data

This standard curve was generated at the Novatein Biosciences laboratory for demonstration purpose only. A standard curve must be run with each assay.



Sensitivity

The sensitivity or minimum detectable dose (MDD) of Mouse Oxytocin was determined to be 17 pg/ml. MDD is defined as the Mouse Oxytocin concentration resulting in an $O.D._{450}$ value that is 2 standard deviations higher than blank.

Spiking and Recovery

Recovery was determined by spiking the following matrices with various concentrations of Mouse Oxytocin.

Sample Type	Average Recovery (%)	Range (%)
Cell lysates	91.1	84-97
Serum	98.3	89-101

Reproducibility

- Inter-assay- <8.8%
- Intra-assay- <6.3%

Specificity

This kit recognizes both natural and recombinant Oxytocin.

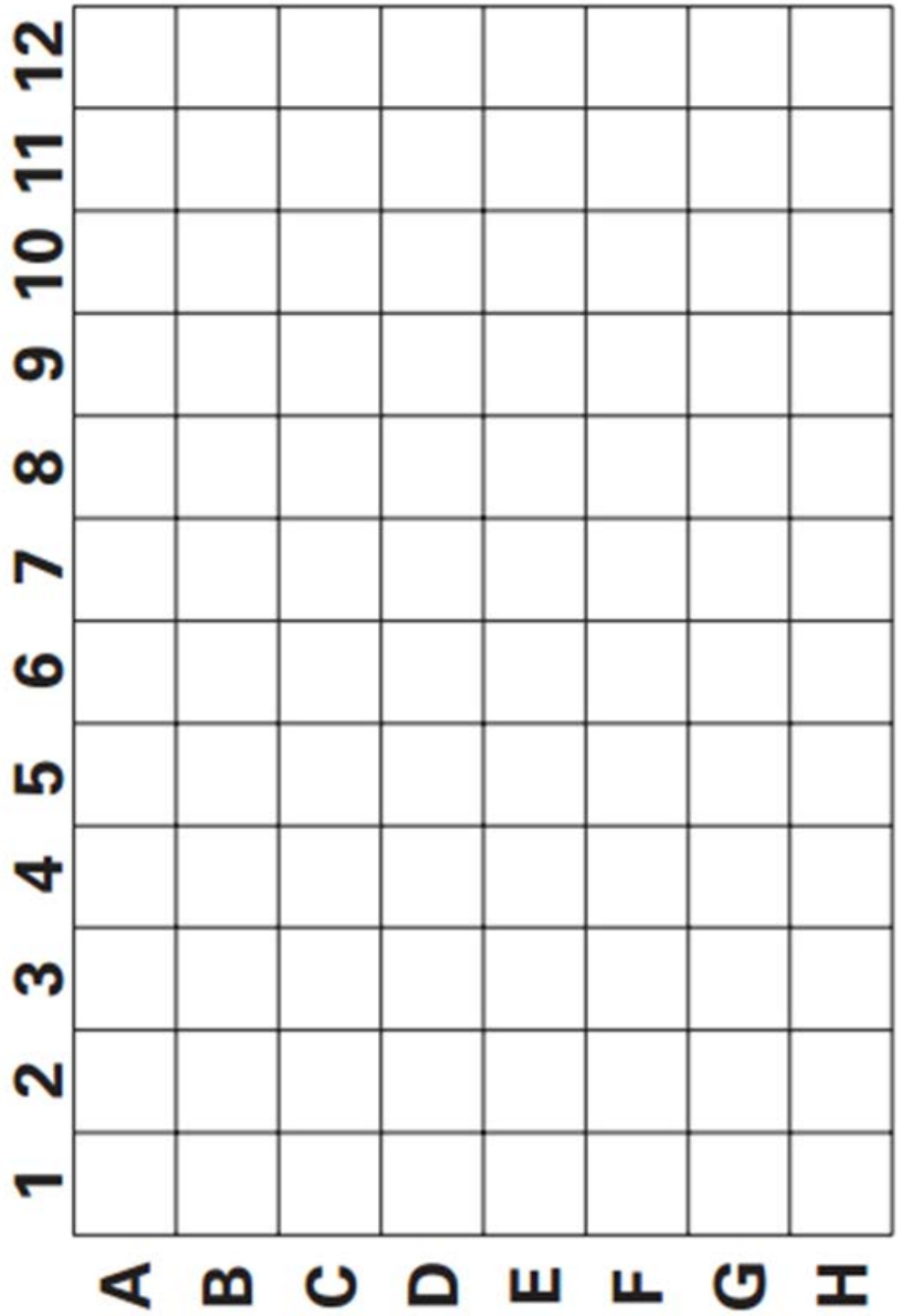
Sample Dilution

The user may need to determine the dilution factor in a preliminary experiment. If required, samples should be diluted in sample diluent buffer.

For trouble shooting information please visit the following website:
http://www.novateinbio.com/en/content/15-tech-info *OR*

Email us at techsupport@novateinbio.com

Plate Layout



Troubleshooting Information

High Background

Probable Cause:	Solution/ Action
High incubation temperature:	Incubate at room temperature (25 °C) throughout the procedure
Insufficient washing of the plate:	Fill the wells with wash buffer and aspirate completely for the next wash Increase the number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, if available and check that all the channels are operating properly
Concentrated streptavidin-HRP	Streptavidin-HRP was not diluted properly Dilute the streptavidin-HRP as mentioned in the manual
Light exposure during substrate incubation	The TMB substrate is light sensitive and turns to blue color in the presence of light. The incubation must be carried out in dark.
Stop solution not added	Color will continue to develop if stop solution is not added
Diluents came with the kit were not used	Standards/ sample, detection antibody and streptavidin-HRP must be diluted in the respective buffers came with the kit. Do not use buffers from other kits
Contaminated solutions	Prepare fresh working solutions

Poor Standard Curve

Probable Cause:	Solution/ Action
Improper standard reconstitution:	Spin the vial briefly before opening Reconstitute the standard as mentioned in the manual. After reconstitution, leave it atleast for 10 minutes at room temperature Do not store and reuse diluted standards
Curve fitting problem:	Log transform the values on both axes Use 4-PL/ 5-PL curve fitting programs
Incubation temperature/ time	Use the recommended standard incubation conditions
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.

No Signal

Probable Cause:	Solution/ Action
Omission of reagent(s):	Read the manual entirely. Check that all the reagents are added in the correct order as stated in the manual
Incorrect detection antibody was used:	Use the detection antibody came with the kit
Chromogen solutions were mixed improperly	Use the recommended procedure to prepare the TMB substrate
HRP inhibitor in sample/ buffers	Check that the samples/ buffers do not have sodium azide as it will inhibit peroxidase reaction.
Vigorous washing	If the washing is done manually, pipette the wash buffer gently.
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations
Improper plate reader settings	Check the wavelength and read the plate again

Erratic duplicate OD values

Probable Cause:	Solution/ Action
Insufficient washing of the plate	Fill the wells with wash buffer and aspirate completely for the next wash Increase number of washes Add soak time (20-30 seconds) in between the washes Use automated plate washer, is available and check that all the channels are functioning properly
Poor dilutions	Pipetting error. Check pipetting technique and calculations. Use calibrated pipettes.
Improper mixing of samples/ buffers	Mix the samples well before pipetting Thoroughly mix the working solutions of detection antibody/ streptavidin-HRP
Contamination from other wells	Do not reuse the adhesive covers from previous assay setups Change pipette tips during reagent addition. If same pipette tip is being used to dispense reagents, care should be taken, not to touch the solution in the well
Precipitates in the samples/ buffer	If precipitates are visible in wash buffer concentrate, keep it at 37 ^{o}C for 10-15 minutes until no precipitates are visible Centrifuge the samples to remove particulate matter
Dried wells	Do not allow the wells to dry out during the assay. Seal with the supplied adhesive cover during incubations