Datasheet for ABIN2815104

Oxytocin CLIA Kit

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Overview

Quantity:	96 tests
Target:	Oxytocin (OXT)
Reactivity:	Various Species
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® Oxytocin Immunoassay kit is designed to quantitatively measure Oxytocin present in serum, plasma, clarified milk and tissue culture media samples.
Brand:	DetectX®
Sample Type:	Serum, Plasma, Milk, Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Chemiluminescent
Cross-Reactivity (Details):	The following cross reactants were tested in the assay and calculated at the 50 % binding point. Steroid Cross Reactivity: Oxytocin 100 %, Isotocin 95.9 %, Mesotocin 88.4 %, Lys8-Vasopressin 0.14 %, Arg8-Vasotocin 0.13 %, Arg8-Vasopressin 0.12 %
Components:	Coated White 96 Well Plates White plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG. 1 Or 5 Each Oxytocin Standard Oxytocin at 50,000 pg/mL in a special stabilizing solution. 125 Or 625 µL DetectX® Oxytocin Antibody A rabbit polyclonal antibody specific for oxytocin. 3 mL Or 13 mL DetectX® Oxytocin Conjugate Oxytocin-peroxidase conjugate in a special stabilizing solution. 3

Product Details

	mL Or 13 mL Assay Buffer Concentrate Assay Buffer, 5X concentrate that should be diluted with deionized or distilled water. 28 mL or 55 mL Extraction Solution A special extraction solution for treatment of serum and plasma samples to extract oxytocin. 50 mL or 250 mL Wash Buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled water. 30 mL Or 125 mL Substrate Solution A 6mL Or 28 mL Substrate Solution B 6mL Or 28 mL Plate Sealer 1 Or 5 Each
Material not included:	Distilled or deionized water. A Speedvac or other centrifugal evaporator, or a manifold and inert gas supply such as nitrogen to evaporate extracted samples. Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25 and 100 µL. A microplate shaker. 96 well microplate reader capable of reading glow chemiluminescence. A list of some models of suitable readers can be found on our website at www.ArborAssays.com/documents/. All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. The number of RLUs obtained is dependant on the sensitivity and gain of the reader used. If you are unsure of how to properly configure your reader contact your plate reader manufacturer or carry out the following protocol: Dilute 5 µL of the Oxytocin Conjugate Concentrate into 45 µL of deionized water. Pipet 5 µL of this dilution into an uncoated white well and add 100 µL of prepared CLIA substrate (see page 8 for details). This well will give you an intensity 2-3 times the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the readers maximum signal. To properly analyze the data software will be required for converting raw RLU readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting.

Target Details

Target:	Oxytocin (OXT)
Alternative Name:	Oxytocin (OXT Products)

Target Details

Target Type:	Hormone
Background:	The neuropeptides, oxytocin and vasopressin were isolated and synthesized by Vincent du Vigneaud at Cor- nel 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 neurotransmitter1,2, recent studies have begun to investigate oxytocin's role in various behav- iors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors3,4 and is important in male reproductive physiology5. Oxytocin and the related neurohypophysial peptide, Arg8-Vasopressin, main- tain renal water and sodium balance6. Oxytocin Highly conserved across species boundaries, oxytocin-like neurohypophysial peptides are substituted primar- ily 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 isoleucine7. 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
Pathways:	Myometrial Relaxation and Contraction, Feeding Behaviour

Application Details

Application Notes:	This assay has been validated for serum, EDTA and heparin plasma, milk, and tissue culture sam- ples. Samples containing visible particulate matter should be centrifuged before use. Oxytocin is identical across all species and we expect this kit may measure oxytocin from sources other than human. Because of the cross reactivity to mesotocin this kit should also be able to measure mesotocin from birds, fish and amphibians. The end user should evaluate recoveries of oxytocin in other samples being tested.
Plate:	Pre-coated
Protocol:	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 white 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 bind- ing 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 substrate is added. The substrate reacts with the bound oxytocin-peroxidase conjugate to produce light. The intensity of the generated chemi- luminescent signal is detected in a microtiter plate reader capable of measuring luminescence. 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.
Reagent Preparation:	Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine oxytocin concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit. Assay Buffer Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deion- ized water. Once diluted this is stable at 4 °C for 3 months. Wash Buffer Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at 4 °C for 3 months. Standard Preparation Label test tubes as #1 through #8. Pipet 450 µL of Assay Buffer into tube #1 and 300 µL into the remaining tubes. The oxytocin stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 50 µL of the oxytocin stock solution to tube #1 and vortex completely. Take 200 µL of the oxytocin solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of oxytocin in tubes 1 through 8 will be 5,000, 2,000, 800, 320, 128, 51.2, 20.48 and 8.192 pg/mL. Use all Standards within 2 hours of preparation.
Sample Preparation:	Serum and plasma samples should be extracted with the provided Extraction Solution, or with a solid phase C18 column extraction protocol prior to running in the kit. Protocol Using Extraction Solution: Mix 1 part sample with 1.5 parts of Extraction Solution. Vortex and then nutate at room temperature for 90 minutes. Centrifuge for 20 minutes at 4 °C at 1660 x g. Speedvac supernatant to dryness at 37 °C. Reconstitute sample with 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.
Assay Procedure:	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 4ºC. 2. Pipet 100 µL of samples or standards into wells in the plate. 3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells. 4. Pipet 100 µL of Assay Buffer into wells to act as maximum binding wells (Bo or 0 pg/mL). 5. Add 25 µL of the DetectX® Oxytocin-Conjugate to each well using a repeater pipet. 6. Add 25 µL of the DetectX® 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 hours. 8. The following day remove the Chemiluminescent 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 mixed Chemiluminescent Substrate to each well, using a repeater pipet. 11. Incubate the plate at room temperature for 5 minutes without shaking. 12. Read the luminescence generated from each well in a mutimode or chemiluminescent plate reader using a 0.1 second read time per well. The chemiluminescent signal will decrease about 40 % over 60 minutes. 13. Use the plate reader's built-in 4PLC software capabilities to calculate oxytocin concentration for each sample.
Calculation of Results:	All luminometers read Relative Light Units (RLU). These RLU readings will vary with make or model of plate reader. Average the duplicate RLU readings for each standard and sample. Cre- ate a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean RLU's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values. Or use the online tool from www.myassays.com/arbor-assays-oxytocin-clia-kit.assay to calculate the data. *The MyAssays logo is a registered trademark of MyAssays Ltd. typical data

Application Details

	Sample Mean RLU Net RLU % B/B0 Oxytocin Conc. (pg/mL) NSB 6,600 0 - - Standard 1 20,735 14,135 9.94 % 5,000 Standard 2 29,455 22,855 16.07 % 2,000 Standard 3 43,100 36,500 25.67 % 800 Standard 4 61,715 55,115 38.76 % 320 Standard 5 91,965 85,365 60.04 % 128 Standard 6 114,750 108,150 76.06 % 51.2 Standard 7 123,370 119,320 83.92 % 20.48 Standard 8 133,855 127,255 89.50 % 8.192 B0 148,785 142,185 100 % 0 Sample 1 32,505 25,905 18.22 % 1,413.4 Sample 2 73,910 67,310 47.34 % 229.1 Always run your own standard curve for calculation of results. Do not use this data. Conversion Factor: 1 ng/mL of oxytocin is equivalent to 0.993 nM.
Assay Precision:	Three samples were diluted with Assay Buffer and run in replicates of 20 in an assay. Inter Assay Precision: Three samples were diluted with Assay Buffer and run in duplicates in 14 assays run over multiple days by four operators.
Restrictions:	For Research Use only

Handling

Preservative:	Sodium azide
Precaution of Use:	As with all such products, this kit should only be used by qualified personnel who have had labo- ratory safety instruction. The complete insert should be read and understood before attempting to use the product. The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly. This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.
Storage:	4 °C,RT
Storage Comment:	This kit should be stored at 4°C until the expiration date of the kit.

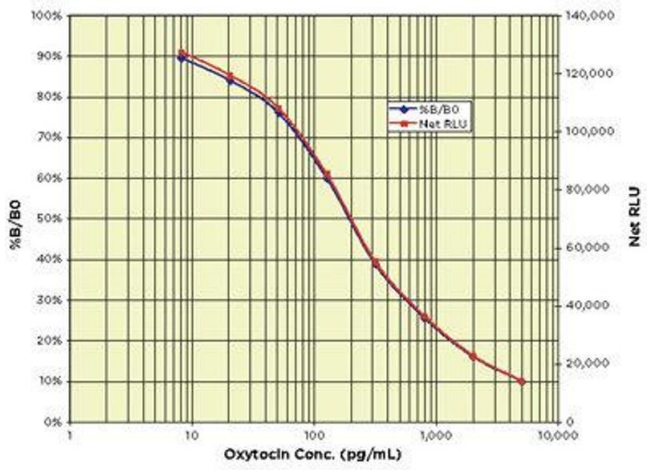

Image 1.

