ARBOR ASSAYS™
Interactive Assay Solutions™

MULTI SPECIES
N-CAL™ KIT

NCal™ International Standard Kit

DetectX®

Oxytocin
Enzyme Immunoassay Kit

1 or 5 Strip Plates	Catalog Number K048-H1/H5
1 or 5 Whole Plates	Catalog Number K048-H1W/H5W

Species Independent

Sample Types Validated:

Serum, Plasma, Saliva, Clarified Milk, and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

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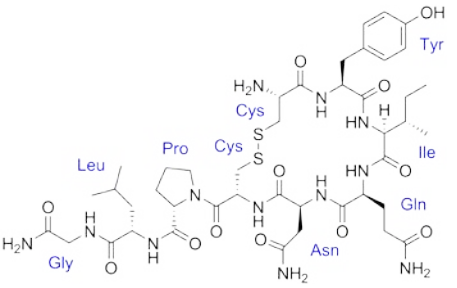
BACKGROUND

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 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 physiology[5]. Oxytocin and the related neurohypophysial peptide, Arg[8]-Vasopressin, maintain renal water and sodium balance[6].



Oxytocin

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.

1. G.L. Kovacs, & D.H.K. Versteeg, "Neurohypophysial Peptides and Brain Neurochemistry." 1993, Ann NY Acad. Sci., 689:7 309-319.
2. Insel TR, Young L, and Wang Z, "Central oxytocin and reproductive behaviours." Rev. Reprod., 1997, 2(1): 28-37.
3. A. Frasch, et al., "Oxytocin: Cellular and Molecular Approaches", 1995, NY: Plenum Press.
4. M.M. McCarthy, & M. Altemus, "Central nervous system actions of oxytocin and modulation of behavior in humans. ", Mol. Med. Today, 1997, 3(6): 269-275.
5. A. Argiolas, & M.R. Melis, "Oxytocin: Cellular and Molecular Approaches", 1995, NY: Plenum Press.
6. K.P. Conrad, et al., "Influence of oxytocin on renal hemodynamics and sodium excretion.", Ann. NY Acad. Sci., 1993, 689: 346-362.
7. R. Archer, & J. Chauvet, "The neurohypophysial endocrine regulatory cascade: precursors, mediators, receptors, and effectors.", Front. Neuroendo., 1995, 16: 237-289.

ASSAY PRINCIPLE

The DetectX® 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.

RELATED PRODUCTS

Kits	Catalog No.
17-Hydroxyprogesterone ELISA Kits	K053-H1/H5
Aldosterone ELISA & Chemiluminescent ELISA Kits	K052-H1/H5, K052-C1/C5
Allopregnanolone ELISA Kits	K061-H1/H5
Ceruloplasmin Colorimetric Activity Kit	K035-H1
Corticosterone Chemiluminescent ELISA Kits	K014-C1/C5
Corticosterone ELISA Kits (Strip Wells and Whole Plate)	K014-H1/H5/H1W/H5W
Cortisol ELISA Kits (Strip Wells and Whole Plate)	K003-H1/H5/H1W/H5W
Cortisone ELISA & Chemiluminescent ELISA Kits	K017-H1/H5, K017-C1/C5
Estradiol Non-Invasive & Serum ELISA Kits	K030-H1/H5, KB30-H1/H5
Estrone ELISA Kits	K031-H1/H5
Estrone-3-Glucuronide (E1G) ELISA Kits	K036-H1/H5
Estrone-3-Sulfate (E1S) ELISA Kit	K038-H1/H5
Levonorgestrel (LNG) ELISA Kits	K058-H1/H5
Oxytocin Chemiluminescent ELISA Kits	K048-C1/C5
PGFM (13,14,Dihydro-15-keto-Prostaglandin F_{2a}) ELISA Kits	K022-H1/H5
Pregnanediol-3-Glucuronide (PDG) ELISA Kits	K037-H1/H5
Progesterone ELISA Kits	K025-H1/H5/H1W/H5W
Progesterone Metabolites ELISA Kits	K068-H1/H5
Prolactin (PRL) ELISA Kit	K040-H1
Isotocin Solution	X128-625UL
Mesotocin Solution	X127-625UL

SUPPLIED COMPONENTS

Coated Clear 96 Well Plates

Clear plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG.

Kit K048-H1 **or** -H5	1 **or** 5 Each	Catalog Number X016-1EA, 1 x 8 Strip Well
Kit K048-H1W **or** -H5W	1 **or** 5 Each	Catalog Number X015-1EA, Whole Well

Oxytocin Standard

Oxytocin at 100,000 pg/mL in a special stabilizing solution.

Kit K048-H1/H1W **or** -H5/H5W	125 µL **or** 625 µL	Catalog Number C167-125UL **or** -625U

Calibrated to the 4th WHO International Standard NIBSC code: 76/575

DetectX® Oxytocin Antibody

A rabbit polyclonal antibody specific for oxytocin.

Kit K048-H1/H1W **or** -H5/H5W	3 mL **or** 13 mL	Catalog Number C165-3ML **or** -13ML

DetectX® Oxytocin Conjugate

Oxytocin-peroxidase conjugate in a special stabilizing solution.

Kit K048-H1/H1W **or** -H5/H5W	3 mL **or** 13 mL	Catalog Number C166-3ML **or** -13ML

Assay Buffer Concentrate

Assay Buffer, 5X concentrate that should be diluted with deionized or distilled water.

Kit K048-H1/H1W **or** -H5/H5W	28 mL **or** 55 mL	Catalog Number X065-28ML **or** -55ML

Extraction Solution

A special extraction solution for treatment of serum and plasma samples to extract oxytocin.

Kit K048-H1/H1W **or** -H5/H5W	50 mL **or** 250 mL	Catalog Number X123-50ML **or** -250ML

Wash Buffer Concentrate

A 20X concentrate that should be diluted with deionized or distilled water.

Kit K048-H1/H1W **or** -H5/H5W	30 mL **or** 125 mL	Catalog Number X007-30ML **or** -125ML

TMB Substrate

Kit K048-H1/H1W **or** -H5/H5W	11 mL **or** 55 mL	Catalog Number X019-11ML **or** -55ML

Stop Solution

A 1M solution of hydrochloric acid. **CAUSTIC**.

Kit K048-H1/H1W **or** -H5/H5W	5 mL **or** 25 mL	Catalog Number X020-5ML **or** -25ML

Plate Sealer

Kit K048-H1/H1W **or** -H5/H5W	1 **or** 5 Each	Catalog Number X002-1EA

STORAGE INSTRUCTIONS

This kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes.

A Speedvac/centrifugal concentrator or N_2 gas and gas manifold for evaporation.

Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25 µL, 50 µL, and 100 µL.

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory 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.

SAMPLE TYPES

This assay has been validated in-house for serum, EDTA and heparin plasma, clarified milk and tissue culture samples using the preparation protocols below. Samples containing visible particulate matter should be centrifuged before use.

Highlighting the importance of validating any assay and sample handling for each species and sample matrix being evaluated, the following publications have cited use of the DetectX® Oxytocin ELISA Kit with alternate sample handling methods for urine and plasma after proper validation.

- G.E. Gnanadesikan, et al., “Specificity of plasma oxytocin immunoassays: A comparison of commercial assays and sample preparation techniques using oxytocin knockout and wildtype mice.” Psychoneuroendocrinology, 2021, Oct; https://doi.org/10.1016/j.psyneuen.2021/105368
- G. Wirobski, F.S. Schaebs, F. Range, et al., “Analytical and physiological validation of an enzyme immunoassay to measure oxytocin in dog, wolf, and human urine samples.” Nature Sci Rep, 2021, 11:12793; https://doi.org/10.1038/s41598-021-92356-z

See additional validation work with our assay at https://www.arborassays.com/product/oxytocin-enzyme-immunoassay-kit/#publications.

Oxytocin is identical across all species that produce it and we expect this kit may measure oxytocin from sources other than human. Due to the cross reactivity profile of the assay to isotocin and mesotocin, this kit will also measure isotocin from fish and mesotocin from birds, amphibians, and reptiles. The end user should evaluate recoveries of oxytocin in other samples being tested.

SAMPLE PREPARATION

Serum and Plasma

Serum and plasma samples should be extracted with the provided Extraction Solution, or with a solid phase C18 column extraction protocol (see Peptide/Protein Extraction Protocol at www.ArborAssays.com/resources/#protocols) 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

Saliva should be collected with Salivettes (https://www.sarstedt.com/en/products/diagnostic/salivasputum/product/51.1534/) or following the "Saliva Sample Handling Protocol" found on our resource page (www.arborassays.com/resources/) of Assay Buffer.

A small number of saliva samples were tested in-house, comparing dilution to extracted using the extraction reagent as described for serum and plasma. Both methods showed parallelism and spiked recovery, with the measured concentration of extracted samples being approximately 60% higher than the measured concentration of samples diluted 1:2-1:8 in the assay buffer. Due to the difference seen, we recommend clearly outlining the chosen methodology and continuing it throughout experimental studies to ensure accurate comparisons between data.

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

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer

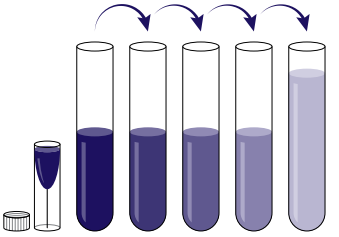
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 room temperature 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 10,000, 4,000, 1,600, 640, 256, 102.4, 40.96 and 16.38 pg/mL.



Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	**450**	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Vol of Addition (µL)	**50**	200	200	200	200	200	200	200
Final Conc (pg/mL)	10,000	4,000	1,600	640	256	102.4	40.96	16.38

ASSAY PROTOCOL

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.
2. If you are using the 1 by 8 well strip plate version of the kit, K048-H1 or -H5, 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.

   Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.

   The use of any wells in the whole plate versions of the kit, K048-H1W and K048-H5W will not allow use of unused parts of that plate in a later assay.
3. Pipet 100 µL of samples or standards into wells in the plate.
4. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
5. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
6. Add 25 µL of the DetectX® Oxytocin Conjugate to each well using a repeater pipet.
7. Add 25 µL of the DetectX® Oxytocin Antibody to each well, **except the NSB wells**, using a repeater pipet.
8. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. We recommend shaking at around 700–900 rpm. Cover the plate with the plate sealer and store at 4°C for 16-18 hours.
9. 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.**
10. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
11. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
12. Incubate the plate at room temperature for 30 minutes without shaking.
13. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
14. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
15. Use the plate reader's built-in 4PLC software capabilities to calculate oxytocin concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean ODs 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 MyAssays to calculate the data:
www.myassays.com/arbor-assays-oxytocin-eia-kit.assay

TYPICAL DATA

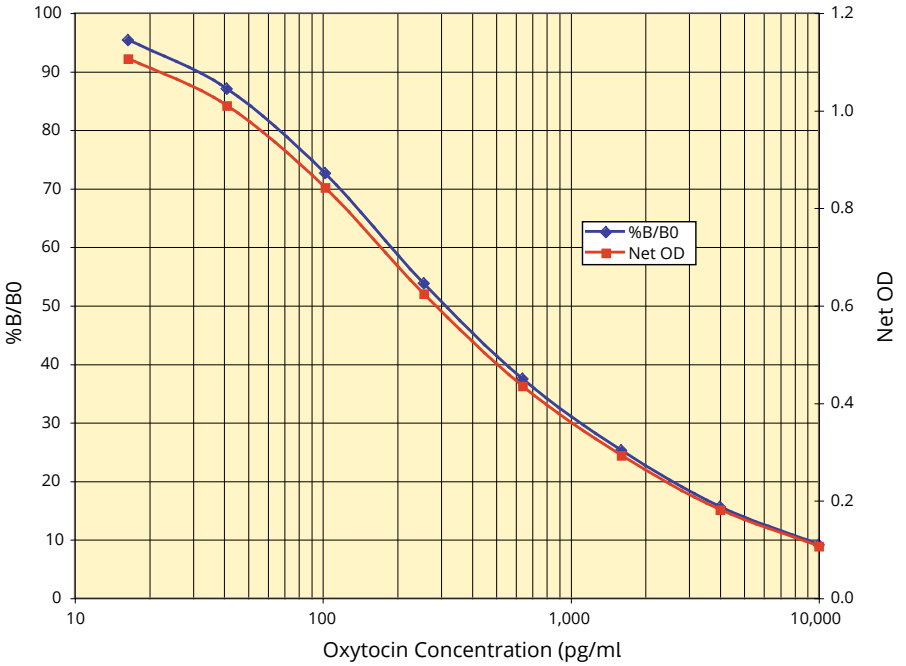
Sample	Mean OD	Net OD	% B/B0	Oxytocin Conc. (pg/mL)
NSB	0.080	0	-	-
Standard 1	0.186	0.106	9.1%	10,000
Standard 2	0.261	0.181	15.6%	4,000
Standard 3	0.373	0.293	25.3%	1,600
Standard 4	0.515	0.435	37.5%	640
Standard 5	0.704	0.624	53.8%	256
Standard 6	0.922	0.842	72.6%	102.4
Standard 7	1.090	1.010	87.1%	40.96
Standard 8	1.186	1.106	95.3%	16.38
B0	1.240	1.160	100%	0
Sample 1	0.380	0.300	25.9%	1,414.5
Sample 2	0.765	0.685	59.0%	206.1

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.

Calibrated to the 4th WHO International Standard NIBSC code: 76/575

Typical Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

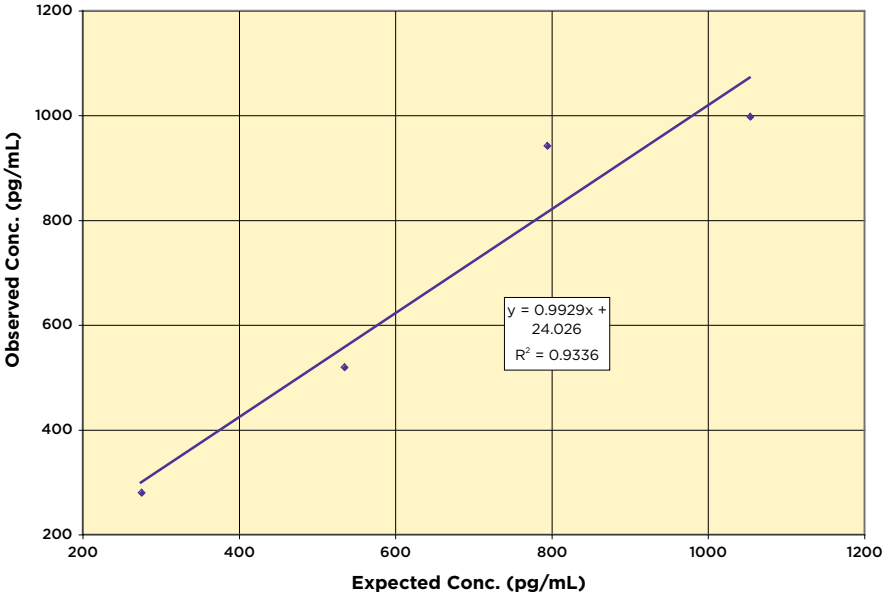
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 17.0 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration sample. **Limit of Detection was determined as 22.9 pg/mL.**

Linearity

Linearity was determined by taking two diluted samples, one with a low oxytocin level of 16.3 pg/mL and one with a higher oxytocin level of 1,313.6 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Sample	Low Sample	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	1,054.2	997.2	94.6%
60%	40%	794.7	941.6	118.5%
40%	60%	535.2	518.8	96.9%
20%	80%	275.7	279.4	101.3%
			Mean Recovery	**102.8%**



Intra Assay Precision

Two samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Oxytocin concentrations were:

Sample	Oxytocin Conc. (pg/mL)	%CV
1	1,391.0	5.2
2	193.8	4.3

Inter Assay Precision

Two samples were diluted with Assay Buffer and run in duplicates in 17 assays run over multiple days by four operators. The mean and precision of the calculated Oxytocin concentrations were:

Sample	Oxytocin Conc. (pg/mL)	%CV
1	1,334.0	7.7%
2	205.7	10.0%

SAMPLE VALUES

Multiple human serum samples were tested in the Chemiluminescent ELISA assay which uses the same antibody and conjugate as the ELISA. Extracted samples were diluted and values ranged from 10.8 to over 70 pg/mL with an average for the samples of 43.02 pg/mL. Average serum levels of oxytocin in monkeys are reported to be 33.6 ± 4.6 pg/mL[8]. Diluted clarified milk samples gave levels of oxytocin of between 657 and 752 pg/mL with an average of 704.2 pg/mL.

In the ELISA assay, 6 serum samples were treated with the Extraction Solution and concentrated to twice the concentration. Sample oxytocin values ranged from 17.03 pg/mL to 38.35 pg/mL with an average value of 25.76 pg/mL.

8. Kawasaki, K., et. al. “Simple method for assaying serum oxytocin and changes of serum oxytocin level during parturition in cynomolgus monkeys”, 2002, Exp. Anim. 51:2, 181-185.

CROSS REACTIVITY

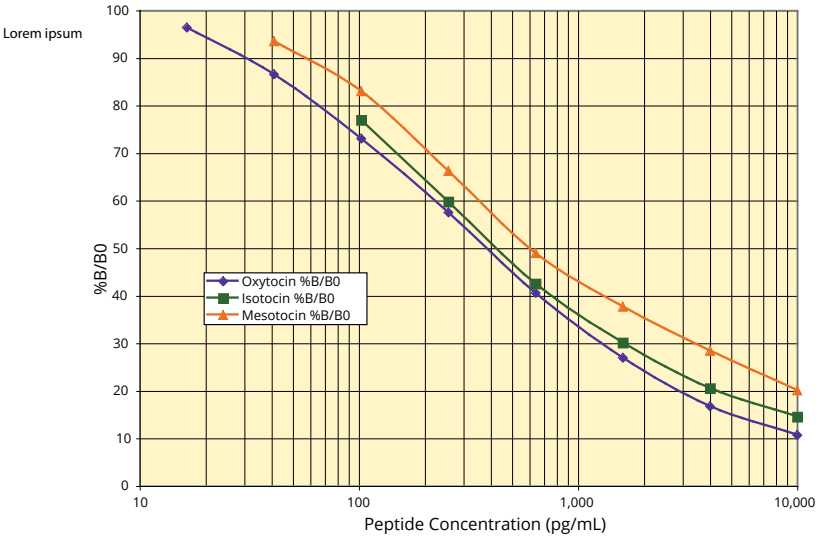
The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Oxytocin	100%
Isotocin	94.3%
Mesotocin	88.4%
Lys^8-Vasopressin	0.14%
Arg^8-Vasotocin	0.13%
Arg^8-Vasopressin	0.12%

PEPTIDE STANDARD CURVES

Oxytocin is produced in the paraventricular nuclei of the hypothalamus in mammals, but in birds, reptiles, amphibians and most marsupials, mesotocin is the expressed form. Isotocin is found primarily in fish.

Mesotocin differs from oxytocin by the substitution of isoleucine for leucine at position 8. Isotocin has a serine replacement for glutamine at position 4. The curves below was generated to allow users to assess the use of isotocin and mesotocin in birds, reptiles, amphibians and most marsupials.



LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

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OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with ELISA kits for wildlife conservation research.

ASSAYS™
FOR SERIOUS SCIENCE

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