

ARBOR ASSAYS™
Interactive Assay Solutions™



NCal™ International Standard Kit
DetectX®
Oxytocin
Chemiluminescent Immunoassay Kit

1 Plate Kit Catalog Number K048-C1

5 Plate Kit Catalog Number K048-C5

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.

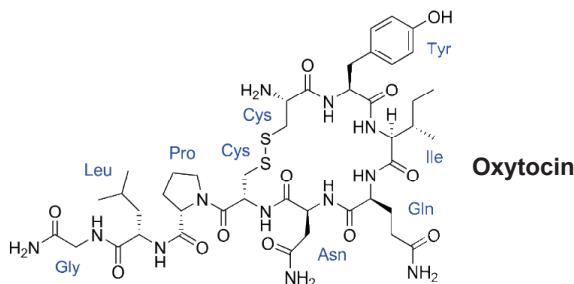
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BACKGROUND

The neuropeptides oxytocin and vasopressin were isolated and synthesized by Vincent du Vigneaud at Cornell 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 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 isoleucine⁷. Acting in classical endocrine fashion, Oxytocin elicits regulatory effects by binding specific cell surface receptors which in turn initiate a secondary intracellular response cascade via a phosphoinositide signaling pathway.

1. G.L. Kovacs, & D.H.K. Versteeg, "Neurohypophysial Peptides and Brain Neurochemistry." Ann NY Acad. Sci., 1993, 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 Chemiluminescent 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 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 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 substrate is added. The substrate reacts with the bound oxytocin-peroxidase conjugate to produce light. The intensity of the generated chemiluminescent 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.

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 ELISA & Chemiluminescent ELISA Kits	K014-H1/H5, K014-C1/C5
Cortisol ELISA Kits	K003-H1/H5
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 ELISA Kits	K048-H1/H5
PGFM (13,14,Dihydro-15-keto-Prostaglandin F2 α) ELISA Kits	K022-H1/H5
Pregnanediol-3-Glucuronide (PDG) ELISA Kits	K037-H1/H5
Progesterone ELISA Kits	K025-H1/H5
Progesterone Metabolites ELISA Kits	K068-H1/H5
Prolactin (PRL) ELISA Kit	K040-H1
Isotocin Solution	X128-625UL
Mesotocin Solution	X127-625UL

SUPPLIED COMPONENTS

Coated White 96 Well Plates

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

Kit K048-C1 or -C5

1 or 5 Each

Catalog Number X014-1EA

Oxytocin Standard

Oxytocin at 50,000 pg/mL in a special stabilizing solution.

Kit K048-C1 or -C5

125 μ L or 625 μ L

Catalog Number C164-125UL or -625UL

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

DetectX® Oxytocin Antibody

A rabbit polyclonal antibody specific for oxytocin.

Kit K048-C1 or -C5

3 mL or 13 mL

Catalog Number C162-3ML or -13ML

DetectX® Oxytocin Conjugate

Oxytocin-peroxidase conjugate in a special stabilizing solution.

Kit K048-C1 or -C5

3 mL or 13 mL

Catalog Number C163-3ML or -13ML

Assay Buffer Concentrate

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

Kit K048-C1 or -C5

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-C1 or -C5

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-C1 or -C5

30 mL or 125 mL

Catalog Number X007-30ML or -125ML

Substrate Solution A

Kit K048-C1 or -C5

6mL or 28 mL

Catalog Number X077-6ML or -28ML

Substrate Solution B

Kit K048-C1 or -C5

6mL or 28 mL

Catalog Number X078-6ML or -28ML

Plate Sealer

Kit K048-C1 or -C5

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 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 μ L and 100 μ L.

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/resources/#general-info. 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 μ L of the Oxytocin Conjugate into 45 μ L of deionized water. Pipet 5 μ L of this dilution into an uncoated white well and add 100 μ L 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. 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. Wirowski, 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 prior to running in the kit. See Peptide/Protein Extraction Protocol at www.ArborAssays.com/resources/#protocols.

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/).

A small number of saliva samples were tested in-house in the DetectX® Oxytocin ELISA, K048-H1, 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 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

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 $\geq 1:10$ 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

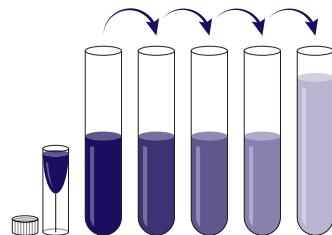
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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 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.

	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)	5,000	2,000	800	320	128	51.2	20.48	8.192

Chemiluminescent Substrate

Mix one part of the Substrate Solution A with one part of Substrate Solution B in a brown bottle. Once mixed the substrate is stable for one month when stored at 4°C.

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. 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 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
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. We recommend shaking at around 700–900 rpm. 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 multimode 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.

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

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. Create 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 MyAssays to calculate the data:

www.myassays.com/arbor-assays-oxytocin-clia-kit.assay

TYPICAL DATA

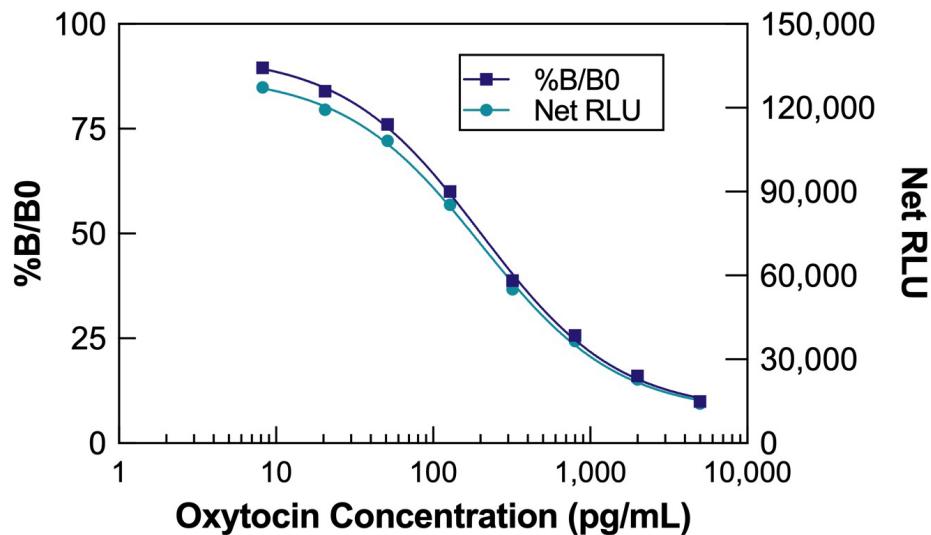
Sample	Mean RLU	Net RLU	% B/B0	Oxytocin Conc. (pg/mL)
NSB	6,600	0	-	-
Standard 1	20,735	14,135	9.94%	5,000
Standard 2	29,455	22,855	16.07%	2,000
Standard 3	43,100	36,500	25.67%	800
Standard 4	61,715	55,115	38.76%	320
Standard 5	91,965	85,365	60.04%	128
Standard 6	114,750	108,150	76.06%	51.2
Standard 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.

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

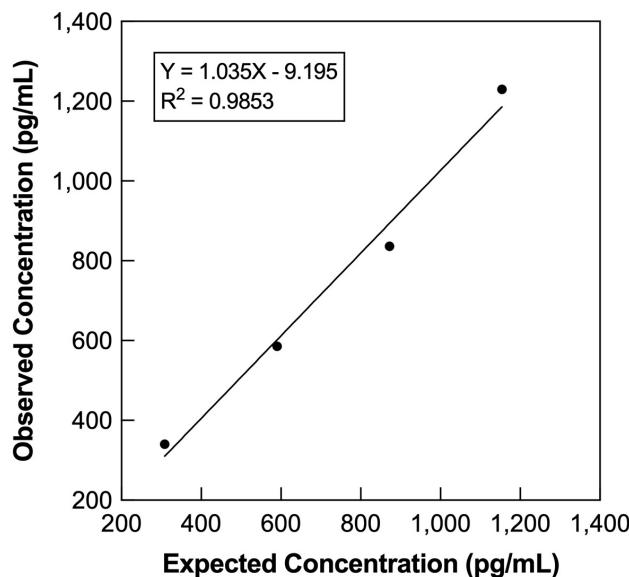
Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 6.33 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the RLU's for twenty runs for each of the zero standard and a low concentration sample. **Limit of Detection was determined as 15.5 pg/mL.**

Linearity

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

High Serum	Low Serum	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	1,154.1	1,229.4	106.5%
60%	40%	872.2	836.6	95.9%
40%	60%	590.4	585.6	99.2%
20%	80%	308.5	340.3	110.3%
Mean Recovery				103.0%



Intra Assay Precision

Three 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	1,265.1	6.4
2	176.8	6.9
3	16.6	28.1

Inter Assay Precision

Three samples were diluted with Assay Buffer and run in duplicates in 14 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	1,350.5	7.4%
2	202.5	8.1%
3	28.8	18.6%

SAMPLE VALUES

Multiple human serum samples were tested in the assay. 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/mL⁸. Diluted clarified milk samples gave levels of oxytocin of between 657 and 752 pg/mL with an average of 704.2 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

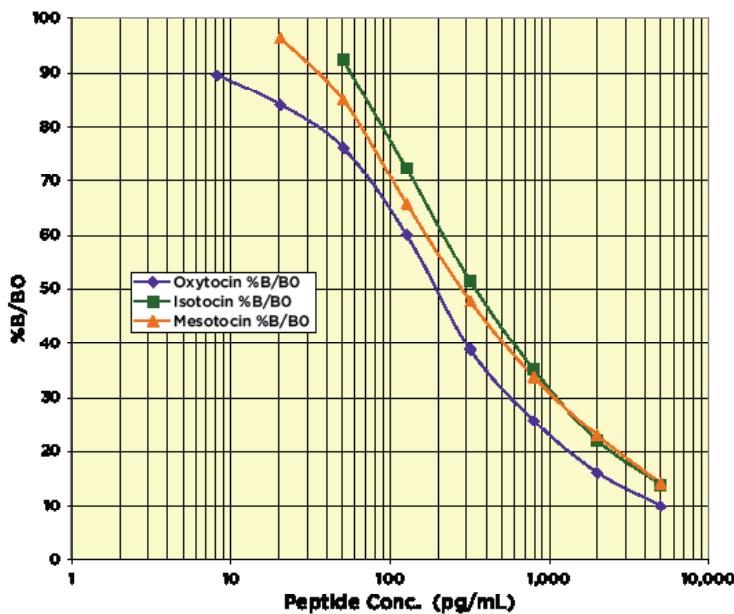
The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Oxytocin	100%
Isotocin	95.9%
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:

Arbor Assays

1143 Highland Dr, Suite A
Ann Arbor, Michigan 48108 USA
Phone: 734-677-1774
Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com
Orders@ArborAssays.com
Technical@ArborAssays.com



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.

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K048-C1/C5