

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.

[www.ArborAssays.com](http://www.ArborAssays.com)   

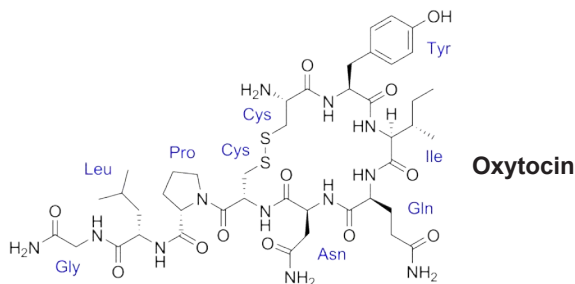
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## BACKGROUND

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 carboxyamided tail. A hormone once thought to be limited to female smooth muscle reproductive physiology and neurotransmitter<sup>1,2</sup>, recent studies have begun to investigate oxytocin's role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors<sup>3,4</sup> and is important in male reproductive physiology<sup>5</sup>. Oxytocin and the related neurohypophysial peptide, Arg<sup>8</sup>-Vasopressin, maintain renal water and sodium balance<sup>6</sup>.



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<sup>7</sup>. 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<sup>®</sup> 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 (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 ELISA Kits  | K048-H1/H5             |
| PGFM (13,14,Dihydro-15-keto-Prostaglandin F2 $\alpha$ ) 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  $\mu$ L or 625  $\mu$ L                      Catalog Number C164-125UL or -625UL  
*Calibrated to the 4th WHO International Standard NIBSC Code: 76/575*

### DetectX<sup>®</sup> 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<sup>®</sup> 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  $\mu$ L and 100  $\mu$ 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](http://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  $\mu$ L of the Oxytocin Conjugate Concentrate into 45  $\mu$ L of deionized water. Pipet 5  $\mu$ L of this dilution into an uncoated white well and add 100  $\mu$ 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 for serum, EDTA and heparin plasma, milk, and tissue culture samples. 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 isotocin and mesotocin this kit should also be able to measure mesotocin from birds, fish, amphibians, and reptiles. The end user should evaluate recoveries of oxytocin in other samples being tested.

## SAMPLE PREPARATION

### Serum and Plasma Samples

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](http://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 Samples

Saliva samples should be extracted using the extraction reagent as described for serum and plasma samples. Saliva should be collected with Salivettes (<https://www.sarstedt.com/en/products/diagnostic/salivasputum/product/51.1534/>), 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  $\geq 1:10$  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

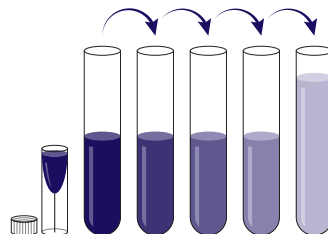
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

### 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  $\mu\text{L}$  of Assay Buffer into tube #1 and 300  $\mu\text{L}$  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  $\mu\text{L}$  of the oxytocin stock solution to tube #1 and vortex completely. Take 200  $\mu\text{L}$  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  $\text{pg/mL}$ .



**Use all Standards within 2 hours of preparation.**

|   | Std 1      | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 | Std 7 | Std 8 |
|---|------------|-------|-------|-------|-------|-------|-------|-------|
| <b>Assay Buffer (<math>\mu\text{L}</math>)</b>    | <b>450</b> | 300   | 300   | 300   | 300   | 300   | 300   | 300   |
| <b>Addition</b>                                   | Stock      | Std 1 | Std 2 | Std 3 | Std 4 | Std 5 | Std 6 | Std 7 |
| <b>Vol of Addition (<math>\mu\text{L}</math>)</b> | <b>50</b>  | 200   | 200   | 200   | 200   | 200   | 200   | 200   |
| <b>Final Conc (<math>\text{pg/mL}</math>)</b>     | 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.



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## 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/arbora-assays-oxytocin-clia-kit.assay](http://www.myassays.com/arbora-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*



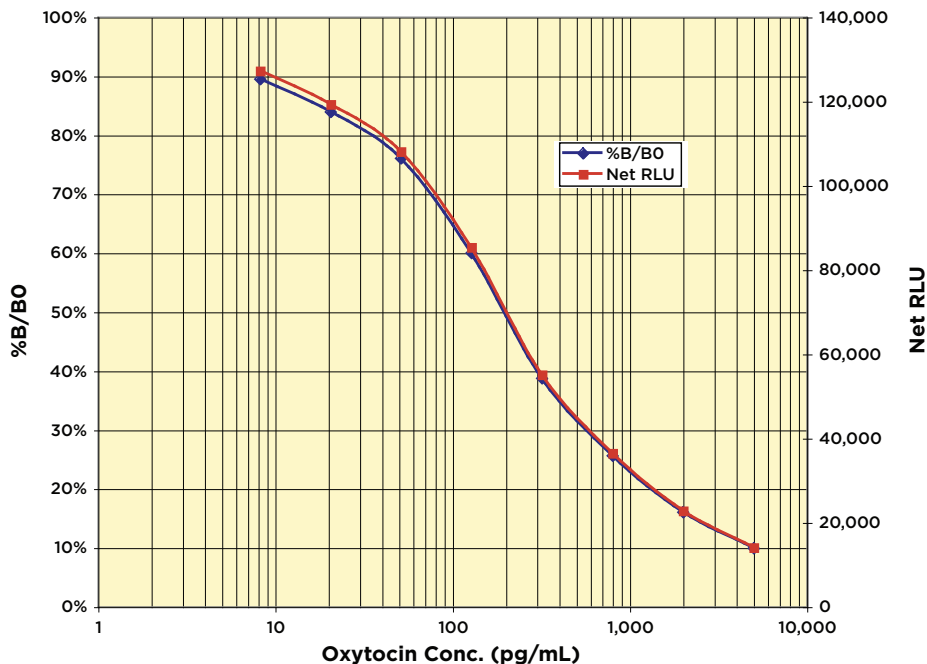
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## Typical Standard Curves



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

## VALIDATION DATA

### Sensitivity and Limit of Detection

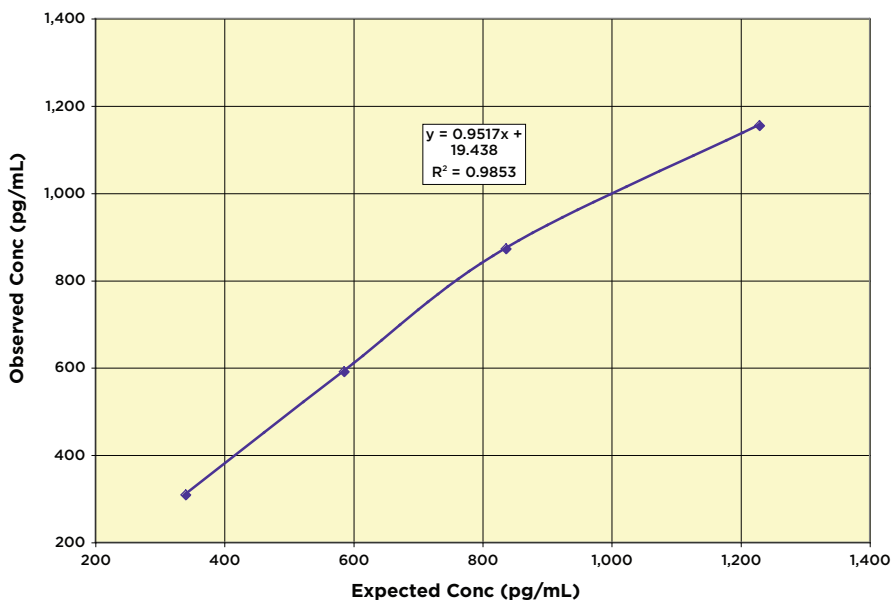
Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 6.33 pg/mL.**

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.<br>(pg/mL) | Observed Conc.<br>(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%        |
| <b>Mean Recovery</b> |           |                           |                           | <b>103.0%</b> |



### 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  $33.6 \pm 4.6$  pg/mL<sup>8</sup>. 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

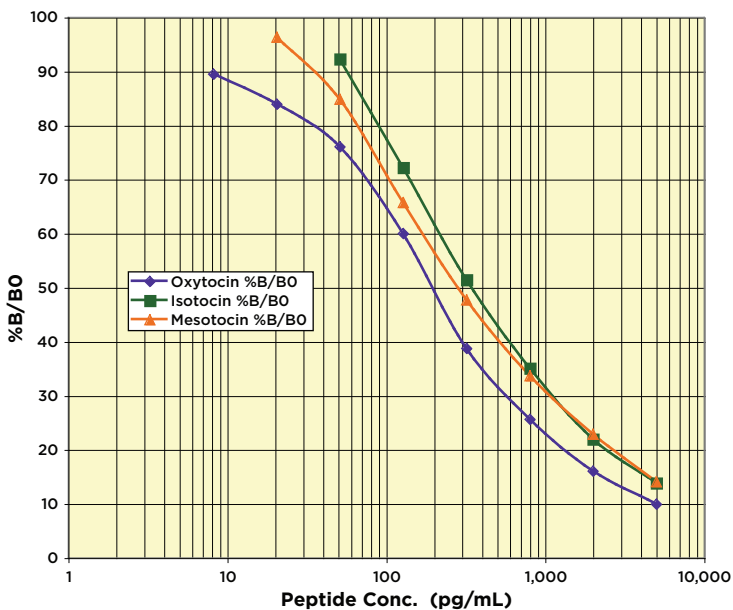
The following cross reactants were tested in the assay and calculated at the 50% binding point.

| Steroid                       | Cross Reactivity (%) |
|-------------------------------|----------------------|
| Oxytocin                      | 100%                 |
| Isotocin                      | 95.9%                |
| Mesotocin                     | 88.4%                |
| Lys <sup>8</sup> -Vasopressin | 0.14%                |
| Arg <sup>8</sup> -Vasotocin   | 0.13%                |
| Arg <sup>8</sup> -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.

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| H | G | F | E | D | C | B | A |    |
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