KAMIYA BIOMEDICAL COMPANY

Oxytocin CLIA Kit

For the quantitative determination of oxytocin in serum, plasma, clarified milk and tissue culture media

Cat. No. KT-735

For Research Use Only.

PRODUCT INFORMATION

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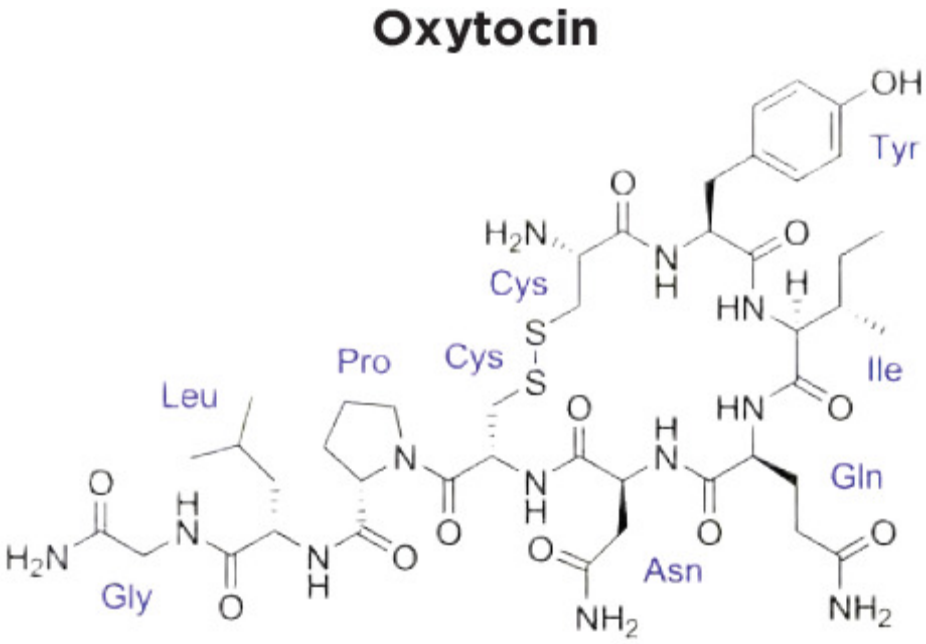
BACKGROUND

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 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, recent studies have begun to investigate oxytocin's role in various behaviors, including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors and is important in male reproductive physiology. Oxytocin and the related neurohypophysial peptide, Arg8-Vasopressin, maintain renal water and sodium balance.



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. 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.

PRINCIPLE

The Oxytocin Immunoassay kit is designed to quantitatively measure Oxytocin present in serum, plasma, clarified milk and tissue culture media samples. Please read the complete kit insert before performing this assay. An oxytocin calibrator is provided to generate a calibration curve for the assay and all samples should be read off the calibration curve. Calibrators 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 calibrators 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℃ 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.

COMPONENTS

Coated White 96 Well Plates
White plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG.
1 Each

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

Oxytocin Antibody
A rabbit polyclonal antibody specific for oxytocin.
3 mL

Oxytocin Conjugate
Oxytocin-peroxidase conjugate in a special stabilizing solution.
3 mL

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

Extraction Solution
A special extraction solution for treatment of serum and plasma samples to extract oxytocin.
50 mL

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL

Substrate Solution A
6mL

Substrate Solution B
6mL

Plate Sealer
1 Each

STORAGE

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

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. 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. 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.

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 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.

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.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. We recommend that all calibrators 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

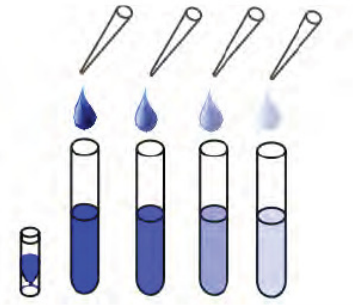
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

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.

Calibrator 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 Calibrators 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℃.

ASSAY PROTOCOL

1. Use the plate layout sheet on the back page to aid in proper sample and calibrator 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 calibrators 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 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℃ for 16 hours.

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

9. Add 100 µL of the mixed Chemiluminescent Substrate to each well, using a repeater pipet.

10. Incubate the plate at room temperature for 5 minutes without shaking.

11. 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.

12. 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 calibrator and sample. Create a calibration 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.

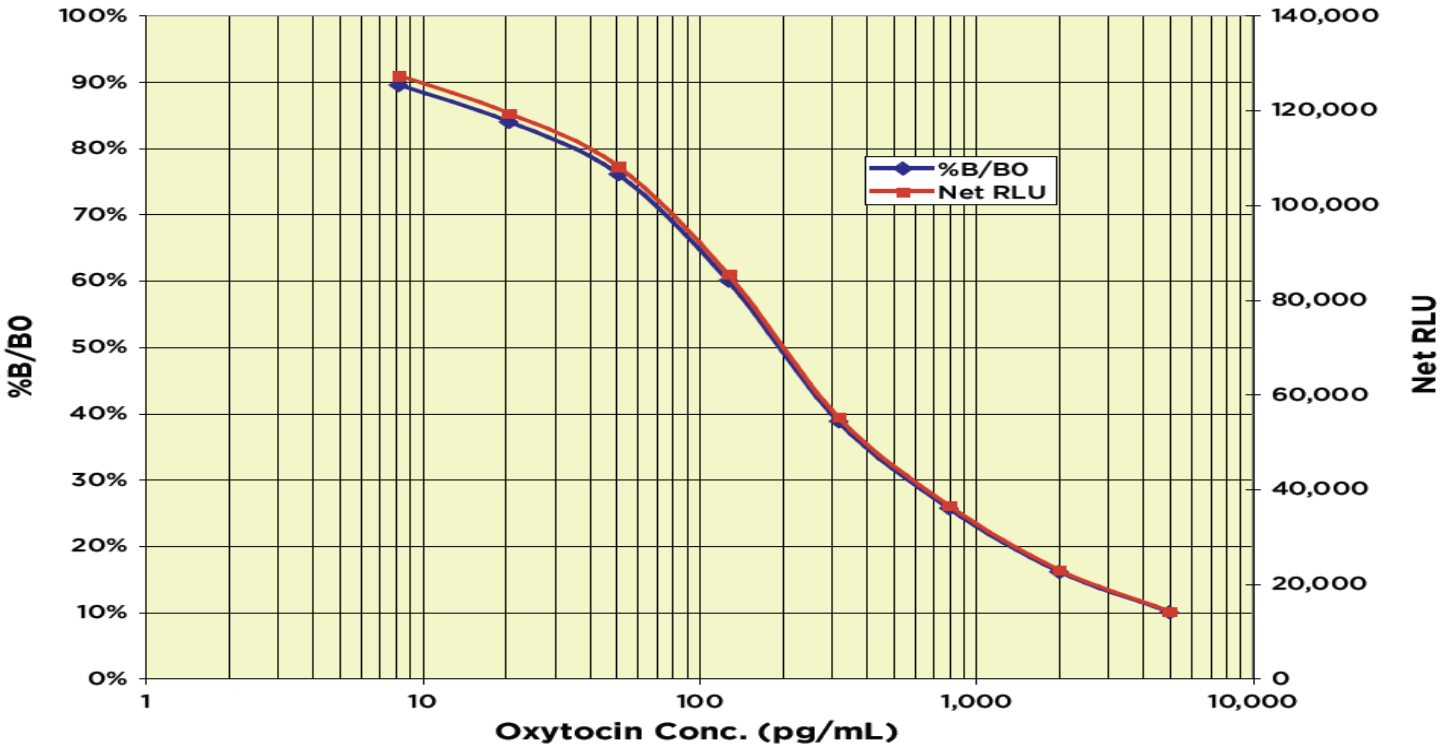
TYPICAL DATA

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 calibration curve for calculation of results. Do not use this data.

Conversion Factor: 1 ng/mL of oxytocin is equivalent to 0.993 nM.

Typical Calibration Curves



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VALIDATION DATA

Sensitivity and Limit of Detection

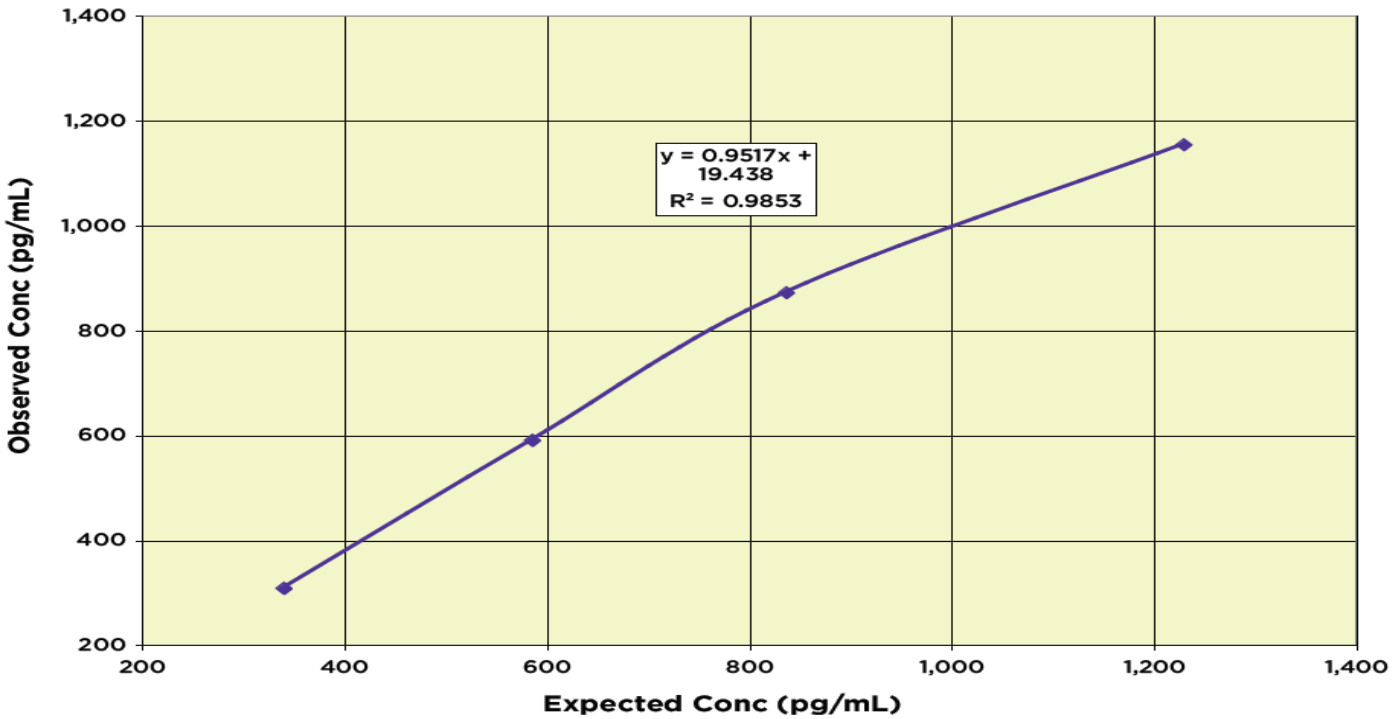
Sensitivity was calculated by comparing the RLU's for twenty wells run for each of the B0 and calibrator #8. The detection limit was determined at two (2) standard deviations from the B0 along the calibration curve.
Sensitivity was determined as 6.33 pg/mL.

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 calibrator 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	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	1,229.4	1,154.1	106.5%
60%	40%	836.6	872.2	95.9%
40%	60%	585.6	590.4	99.2%
20%	80%	340.3	308.5	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/mL. Diluted clarified milk samples gave levels of oxytocin of between 657 and 752 pg/mL with an average of 704.2 pg/mL.

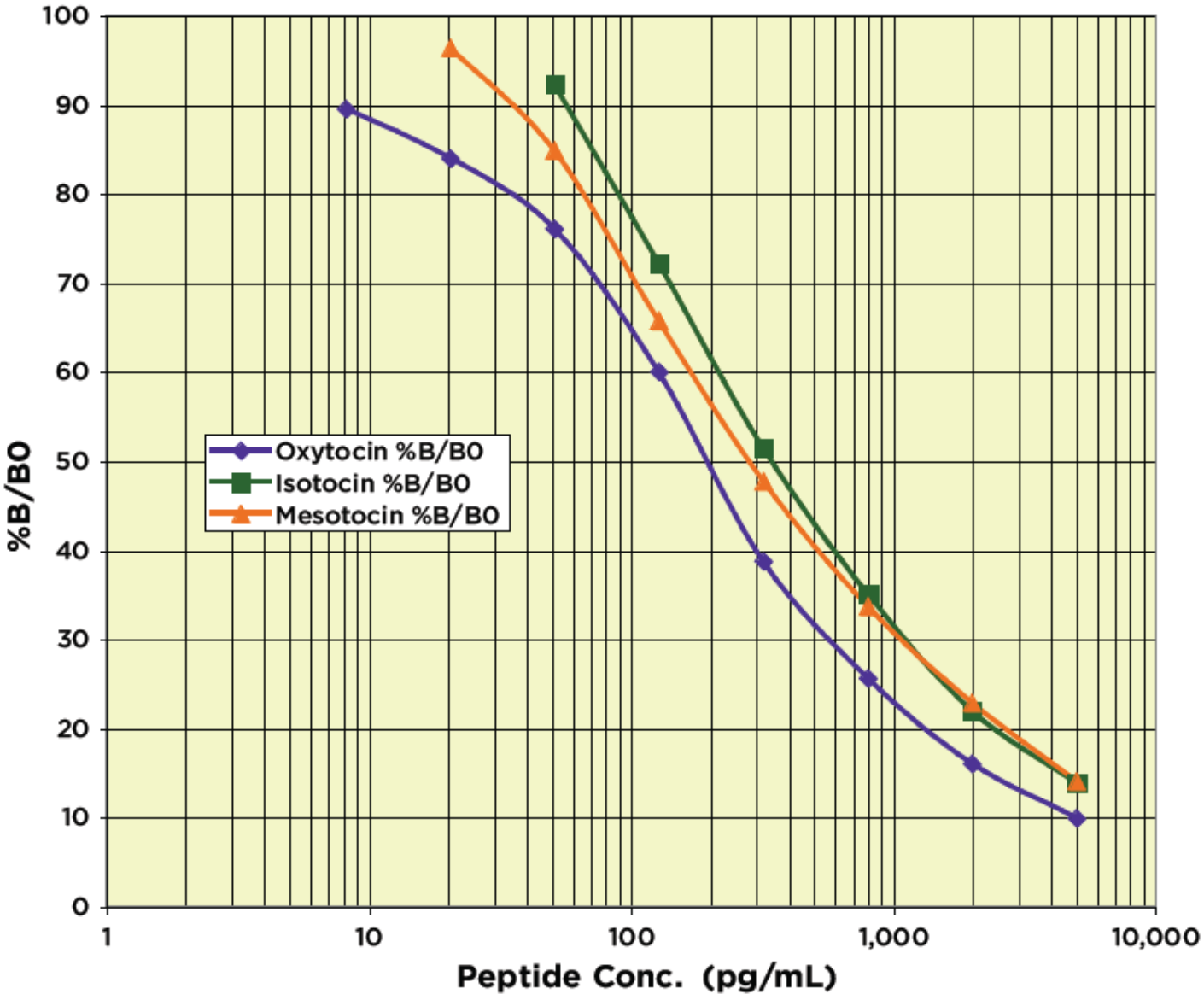
CROSS REACTIVITY

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 CALIBRATION 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.



FOR RESEARCH USE ONLY

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