

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



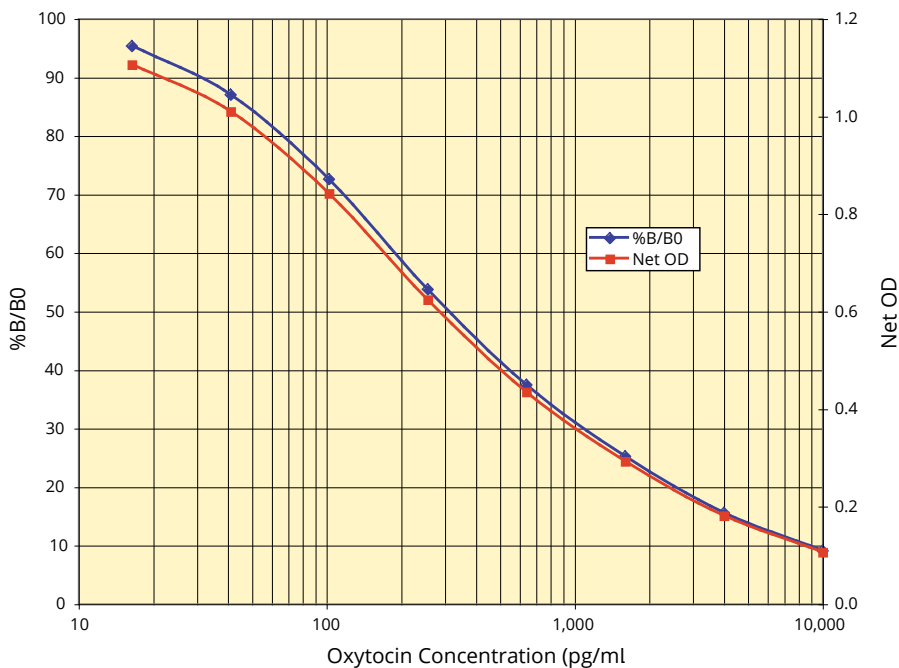
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ASSAYS

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EXPECT ASSAY ARTISTRY™

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

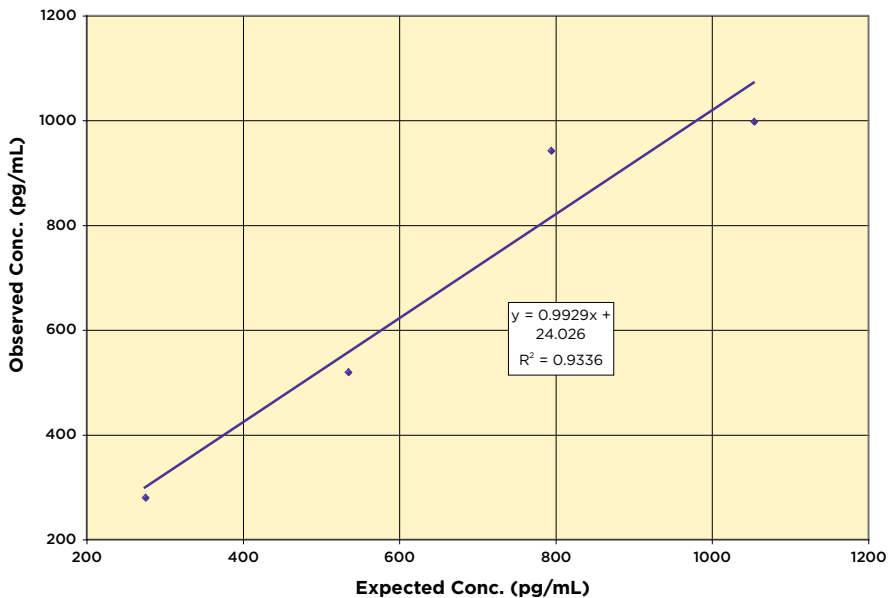
Sensitivity was calculated by comparing the OD's for twenty wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve. **Sensitivity was determined as 17.0 pg/mL.**

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/mL⁸. 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

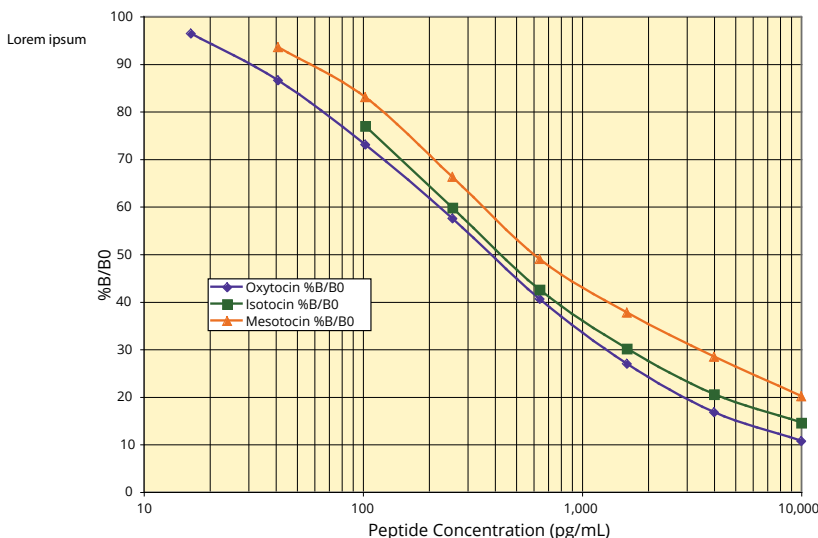
The following cross reactants were tested in the assay and calculated at the 50% binding point.

| 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

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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.

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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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