Oxytocin ELISA Kit

08/17

(Catalog # E4348-100, 100 assays, Store at 4°C)

I. Introduction:

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. BioVision's Oxytocin ELISA kit is a competitive ELISA assay for the quantitative measurement of Oxytocin in extracted serum and plasma, or in urine, extracted dried fecal samples, and tissue culture media samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Oxytocin.
Detection Range: 10000 – 16.38 pg/ml
Sensitivity: < 17 pg/ml
Detection Limit: 22.9 pg/ml

III. Specificity:

Universal

IV. Sample Type:

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

V. Kit Contents:

Components	E4348-100	Part No.
Micro ELISA Plate	8 X 12 strips	E4348-100-1
Standard	125 µl	E4348-100-2
Oxytocin Antibody	3 ml	E4348-100-3
Oxytocin Conjugate	3 ml	E4348-100-4
Assay Buffer Concentrate (5X)	28 ml	E4348-100-5
Extraction Solution	50 ml	E4348-100-6
Wash Buffer Concentrate (20X)	30 ml	E4348-100-7
TMB Substrate	11 ml	E4348-100-8
Stop Solution	5 ml	E4348-100-9
Plate Sealer	1	E4348-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Ethyl acetate or ethanol for serum, plasma or fecal extracts
- Speedvac for evaporation of ethanol or ethyl acetates
- Precision pipettes with disposable tips

VII. Storage and Handling:

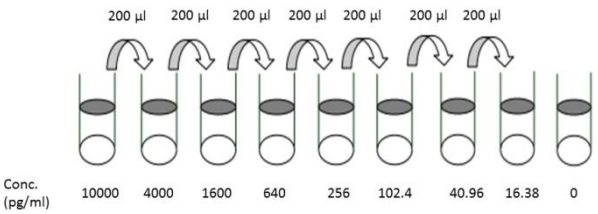
The entire kit may be stored at 4°C for up to 6 months. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **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.
2. **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 for 3 months at room temperature.
3. **Standard Preparation:**

- Add 50 µl of the Oxytocin stock solution to 450 µl of Assay Buffer (tube #1) and vortex completely.
- Prepare 6 vials of standards (tube #2-8) by adding 200 µl of the above stock solution in 300 µl of Assay Buffer. Perform serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 10000, 4000, 1600, 640, 256, 102.4, 40.96, 16.38 pg/ml.



FOR RESEARCH USE ONLY! Not to be used on humans.

- Use all Standards within 2 hours of preparation.

4. Sample Preparation:

Note: Use all Samples within 2 Hours of preparation, or stored at ≤ -20°C until assaying. Avoid multiple freeze-thaw cycles.

- **Extracted serum and plasma**: Mix 1 part sample with 1.5 parts of Extraction Solution.Vortex and then incubate at room temperature for 90 minutes. Centrifuge for 20 minutes at 4°C at 1660 x g. Transfer supernatant to a clean tube. Speedvac supernatant to dryness at 37°C. Reconstitute sample with 250 µl of Assay Buffer.
- **Saliva:** Saliva samples should be extracted using the extraction reagent as described for serum and plasma samples. Saliva should be collected with Sarstedt Salivettes, extracted, dried, and reconstituted in 250 µl of Assay Buffer.
- **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 ≥ 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 End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. Pipet 100 µl of samples or standards into wells in the plate. Pipet 125 µl of Assay Buffer into the non-specific binding (NSB) wells.
3. Add 25 µl of the Oxytocin Conjugate to each well. Add 25 µl of the Oxytocin Antibody to each well, except the NSB wells.
4. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at 4°C for 16-18 hours.
5. The following day, remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes
6. Aspirate the plate and wash each well 4 times with 300 µl wash buffer. Tap the plate dry on clean absorbent towels.
7. Add 100 µl of the TMB Substrate to each well. Incubate the plate at room temperature for 30 minutes without shaking.
8. Add 50 µl of the Stop Solution to each well.
9. Read the optical density at 450 nm within 15 minutes.

X. CALCULATION:

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 OD's for the non-specific binding well (NSB). The sample concentrations obtained, calculated from the $\%B/B_0$ curve, and should be multiplied by the dilution factor to obtain neat sample values.

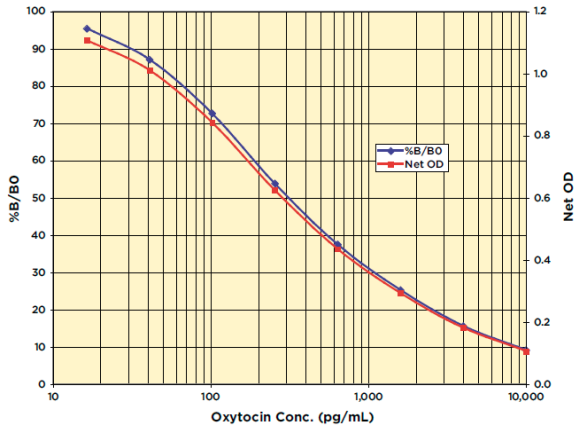


Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. VALIDATION DATA:

Recovery Rate:

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%**

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Intra Assay:

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

Inter Assay Precision:

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

Cross Reactivity:

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%

XII. RELATED PRODUCTS:

- Leucine Aminopeptidase (LAP) Activity Assay Kit (Fluorometric) (Cat. No. K534)
- PF-3274167 (Cat. No. B1026)
- Penicillide (Cat. No. B1245)