Oxytocin ELISA Kit

Item No. 500440

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size	480 wells Quantity/Size
400442	Oxytocin Polyclonal Antiserum	1 vial/100 dtn	1 vial/500 dtn
400440	Oxytocin AChE Tracer	1 vial/100 dtn	1 vial/500 dtn
400444	Oxytocin ELISA Standard	1 vial	1 vial
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml	4 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	1 vial/12.5 ml
400035	Polysorbate 20	1 vial/3 ml	1 vial/3 ml
400004/400006	Mouse Anti-Rabbit IgG Coated Plate	1 plate	5 plates
400012	96-Well Cover Sheet	1 cover	5 covers
400050	Ellman's Reagent	3 vials/100 dtn	6 vials/250 dtn
400040	ELISA Tracer Dye	1 vial	1 vial
400042	ELISA Antiserum Dye	1 vial	1 vial

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Oxytocin ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

When compared to quantification by LC/MS or GC/MS, it is not uncommon for immunoassays to report higher analyte concentrations. While LC/MS or GC/MS analyses typically measure only a single compound, antibodies used in immunoassays sometimes recognize not only the target molecule, but also structurally related molecules, including biologically relevant metabolites. In many cases, measurement of both the parent molecule and metabolites is more representative of the overall biological response than is the measurement of a short-lived parent molecule. It is the responsibility of the researcher to understand the limits of both assay systems and to interpret their data accordingly.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -20°C and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance between 405-420 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials used for **Sample Preparation** (see page 13).

INTRODUCTION

Background

Oxytocin is a nine amino acid hypothalamic peptide hormone that is stored in the posterior pituitary gland.[1] In females, oxytocin is released from the pituitary into the bloodstream in larger quantities during parturition and lactation, facilitating uterine contraction and milk letdown reflex.[1,2] In both males and females, oxytocin is involved in social and sexual behavior and may play a role in neuropsychiatric disorders like autism and postpartum depression.[3-6] Oxytocin and the related hormone arginine vasopressin are involved in maintaining water and sodium homeostasis.

The actions of oxytocin are mediated by stimulation of a tissue-specific G protein-coupled receptor (OXTR) expressed in myoepithelial cells, mammary gland, both myometrium and endometrium of the uterus, and also in the central nervous system.[1] In some mammals, oxytocin receptors are also found in the kidney and heart.[1]

Reported levels of oxytocin in plasma vary depending on the method of measurement (ELISA, RIA, and LC-MS).[7,8] Most common reported levels for ELISA are 1-250 pg/ml.

About This Assay

Cayman's Oxytocin ELISA Kit is a competitive assay that can be used for quantification of oxytocin in plasma, urine, and saliva. The assay has a range from 5.9-750 pg/ml and a sensitivity (80% B/B_0) of approximately 20 pg/ml.

Principle Of This Assay

This assay is based on the competition between oxytocin and a Oxytocin-acetylcholinesterase (AChE) conjugate (Oxytocin Tracer) for a limited amount of Oxytocin Polyclonal Antiserum. Because the concentration of the Oxytocin Tracer is held constant while the concentration of oxytocin varies, the amount of Oxytocin Tracer that is able to bind to the Oxytocin Polyclonal Antiserum will be inversely proportional to the concentration of oxytocin in the well. This antiserum-oxytocin complex binds to mouse monoclonal anti-rabbit IgG that has been previously attached to the well. The plate is washed to remove any unbound reagents and then Ellman's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of Oxytocin Tracer bound to the well, which is inversely proportional to the amount of free oxytocin present in the well during the incubation; or

$$\text{Absorbance} \propto [\text{Bound Oxytocin Tracer}] \propto 1/[\text{Oxytocin}]$$

A schematic of this process is shown in Figure 1, on page 9.

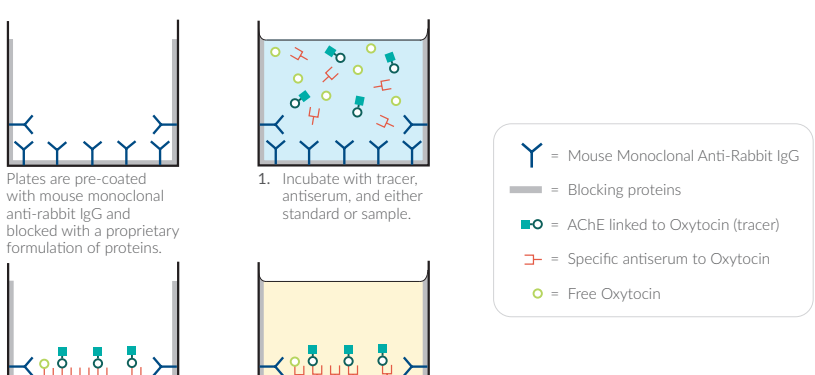


Figure 1. Schematic of the AChE ELISA

Definition of Key Terms

Blank: background absorbance caused by Ellman's Reagent. The blank absorbance should be subtracted from the absorbance readings of all the other wells, including NSB wells.

Total Activity: total enzymatic activity of the AChE-linked tracer. This is analogous to the specific activity of a radioactive tracer.

NSB (Non-Specific Binding): non-immunological binding of the tracer to the well. Even in the absence of specific antibody a very small amount of tracer still binds to the well; the NSB is a measure of this low binding. Do not forget to subtract the Blank absorbance values.

B_0 (Maximum Binding): maximum amount of the tracer that the antibody can bind in the absence of free analyte.

%B/B_0 (%Bound/Maximum Bound): ratio of the absorbance of a particular sample or standard well to that of the maximum binding (B_0) well.

Standard Curve: a plot of the %B/B_0 values *versus* concentration of a series of wells containing various known amounts of analyte.

Dtn: determination, where one dtn is the amount of reagent used per well.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration, two standard deviations away from the mean zero value.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all ELISA reagents and buffers must be deionized and free of trace organic contaminants ('UltraPure'). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for ELISA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months.

1. **ELISA Buffer Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer Concentrate (400X) (96-well kit; Item No. 400062): Dilute to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035).

   OR

   12.5 ml vial Wash Buffer Concentrate (400X) (480-well kit; Item No. 400062): Dilute to a total volume of 5 liters with UltraPure water and add 2.5 ml of Polysorbate 20 (Item No. 400035).

Smaller volumes of Wash Buffer can be prepared by diluting the Wash Buffer Concentrate 1:400 and adding Polysorbate 20 (0.5 ml/liter of Wash Buffer).

NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

Testing for Interference

This assay has been validated using human plasma, urine, and saliva. Other sample types should be tested for interference to evaluate the need for sample purification before embarking on an large number of sample measurements. To test for interference, dilute one or two test samples to obtain at least two different dilutions of each sample between approximately 300 pg/ml to 25 pg/ml (*i.e.*, between 25-76% B/Bo, which is the linear portion of the standard curve). If two different dilutions of the same sample show good correlation (differ by 20% or less) in the final calculated oxytocin concentration, purification is not required. If you do not see good correlation between different dilutions, purification is advised. Purification methods will be determined by the end user and tested for compatibility in the assay. Do not use aprotinin in plasma collection as it causes interference with the assay. Any other protease inhibitors should be tested for the interference with this ELISA prior to use. For best results, we recommend performing purification and the assay immediately after plasma collection.

Plasma Purification

1. Aliquot an equal amount of each plasma sample into clean test tubes (1 ml is recommended).
2. Activate a 6 ml SPE Cartridge (C-18) (6 ml) (Item No. 400020) by rinsing with 2 ml methanol and then 5 ml UltraPure water. Do not allow the SPE Cartridge to dry.
3. Pass the sample through the SPE Cartridge.
4. Wash the column with 6-10 ml of water and then with 3 ml of 3% acetone in water (3 ml of acetone added to 97 ml of water). Let the cartridge air dry for 10-15 minutes or use vacuum drying for 2-3 minutes.
5. Elute oxytocin using two applications of 1 ml of 98% acetone in water (98 ml of acetone added to 2 ml of water).
6. Dry the samples by vacuum centrifugation or under a gentle stream of nitrogen. It is imperative that all the solvent be removed as even trace quantities can affect the ELISA.
7. Resuspend the extract in 1 ml of ELISA Buffer, vortex, and perform the assay. If necessary, samples can be concentrated in this step by resuspending in 0.5 ml of ELISA Buffer, which will result in two-fold concentration. This must be taken into the account when calculating your results.

Sample Matrix Properties

Linearity

To assess dilutional linearity, human plasma and urine were spiked with 300 pg/ml oxytocin; human saliva was spiked with 150 pg/ml of oxytocin. The plasma sample was purified as described in the Sample Preparation section. All samples were serially diluted with ELISA buffer (1x), and evaluated for linearity using the Oxytocin ELISA kit. The results are shown in the table below.

Dilution Factor	Concentration (pg/ml)	Dilutional Linearity (%)
Plasma		
2	303.6	100
4	319.1	105
8	348.2	115
Urine		
2	530.4	100
4	518.5	98
8	530.7	100
Saliva		
2	133.7	100
4	134.1	100
8	157.6	118

Table 1. Dilutional linearity of human plasma, urine, and saliva samples

Spike and Recovery

Pooled plasma and saliva samples and an individual urine sample from a pregnant female were spiked with different amounts of oxytocin. Plasma samples were purified as described in the Sample Preperation section. All samples were serially diluted with ELISA buffer (1X), and analyzed using Oxytocin ELISA kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

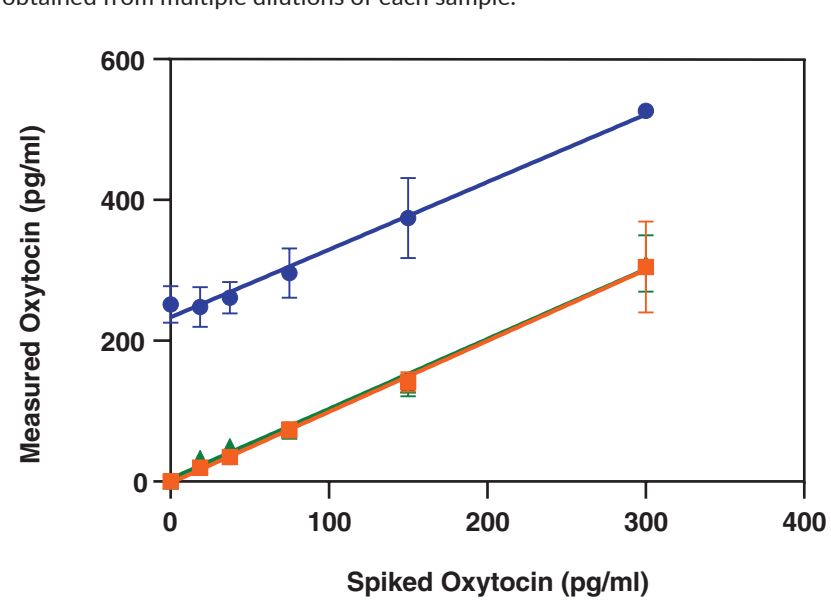


Figure 2. Spike and recovery of Oxytocin in plasma, urine, and saliva

Parallelism

To assess parallelism, spiked and purified human plasma, spiked human saliva and unspiked urine samples were serially diluted and tested using Oxytocin ELISA kit. Measured concentrations were plotted as a function of sample dilution. The results are shown below.

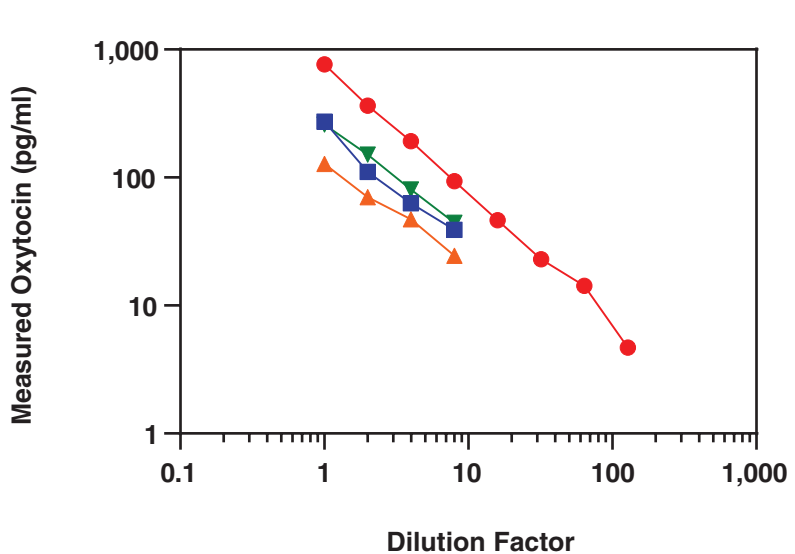


Figure 3. Parallelism in Oxytocin ELISA

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

Oxytocin ELISA Standard

Reconstitute the contents of the Oxytocin ELISA Standard (Item No. 400444) with 1.0 ml of UltraPure water. The concentration of this solution (the bulk standard) will be 7.5 ng/ml. This standard will be stable at 4°C for one week. We recommend storing it at -20°C in small aliquots (100 µl) to use on different days.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and number them #1 through #8. Aliquot 900 µl ELISA Buffer to tube #1 and 500 µl ELISA Buffer to tubes #2-8. Transfer 100 µl of the bulk standard (7.5 ng/ml) to tube #1 and mix thoroughly. Serially dilute the standard by removing 500 µl from tube #1 and placing in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-8. The diluted standards should be used within one hour.

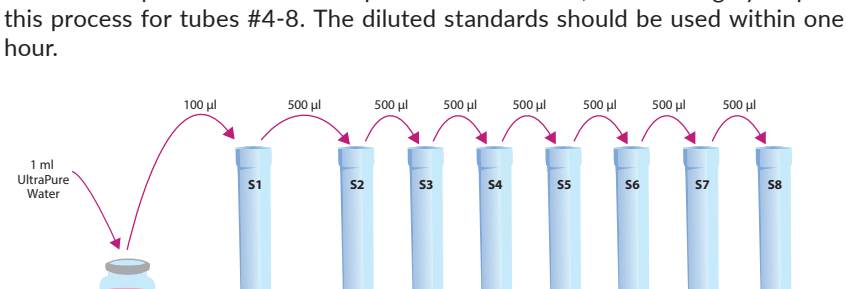


Figure 4. Preparation of the oxytocin standards

Oxytocin AChE Tracer

Reconstitute the Oxytocin AChE Tracer as follows:

100 dtn Oxytocin AChE Tracer (96-well kit; Item No. 414010): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn Oxytocin AChE Tracer (480-well kit; Item No. 414010): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted Oxytocin AChE Tracer at 4°C *(do not freeze!)* and use within four weeks. A 20% surplus of tracer has been included to account for any incidental losses.

Tracer Dye Instructions (optional)

This dye may be added to the tracer, if desired, to aid in visualization of tracer-containing wells. Add the dye to the reconstituted tracer at a final dilution of 1:100 (add 60 µl of dye to 6 ml tracer or add 300 µl of dye to 30 ml of tracer). *NOTE: Do not store tracer with dye for more than 24 hours.*

Oxytocin Polyclonal Antiserum

Reconstitute the Oxytocin Polyclonal Antiserum as follows:

100 dtn Oxytocin Polyclonal Antiserum (96-well kit; Item No. 414013): Reconstitute with 6 ml ELISA Buffer.

OR

500 dtn Oxytocin Polyclonal Antiserum (480-well kit; Item No. 414013): Reconstitute with 30 ml ELISA Buffer.

Store the reconstituted Oxytocin Polyclonal Antiserum at 4°C. It will be stable for at least four weeks. A 20% surplus of antiserum has been included to account for any incidental losses.

Antiserum Dye Instructions (optional)

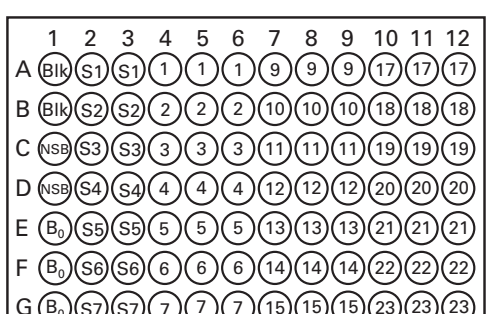
This dye may be added to the antiserum, if desired, to aid in visualization of antiserum-containing wells. Add the dye to the reconstituted antiserum at a final dilution of 1:100 (add 60 µl of dye to 6 ml antiserum or add 300 µl of dye to 30 ml of antiserum). *NOTE: Do not store antiserum with dye for more than 24 hours.*

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *NOTE: If you do not need to use all the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain a minimum of two Blk wells, two NSB wells, two B_0 wells, and an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 5, on page 21. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 21, for more details). We suggest you record the contents of each well on the template sheet provided (see page 33).



Blk - Blank
TA - Total Activity
NSB - Non-Specific Binding
B_0 - Maximum Binding
S1-S8 - Standards 1-8
1-24 - Samples

Figure 5. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of the Reagents

1. **ELISA Buffer**

   Add 150 µl ELISA Buffer to NSB wells. Add 100 µl ELISA Buffer to B_0 wells.

2. **Oxytocin ELISA Standard**

   Add 100 µl from tube #8 to both of the lowest standard wells (S8). Add 100 µl from tube #7 to each of the next two standard wells (S7). Continue with this procedure until all the standards are aliquoted. The same pipette tip should be used to aliquot all the standards. Before pipetting each standard, be sure to equilibrate the pipette tip in that standard.

3. **Samples**

   Add 100 µl of sample per well. Each sample should be assayed at a minimum of two dilutions. Each dilution should be assayed in duplicate (triplicate recommended).

4. **Oxytocin AChE Tracer**

   Add 50 µl to each well *except* the TA and the Blk wells.

5. **Oxytocin Polyclonal Antiserum**

   Add 50 µl to each well *except* the TA, the NSB, and the Blk wells.

Well	ELISA Buffer	Standard/Sample	Tracer	Antiserum
Blk	-	-	-	-
TA	-	-	5 µl (at devl. step)	-
NSB	150 µl	-	50 µl	-
B_0	100 µl	-	50 µl	50 µl
Std/Sample	-	100 µl	50 µl	50 µl

Table 2. Pipetting summary

Incubation of the Plate

Cover each plate with plastic film (Item No. 400012) and incubate overnight (approximately 18 hours) at 4°C.

Development of the Plate

1. Reconstitute Ellman's Reagent immediately before use (20 ml of reagent is sufficient to develop 100 wells):

   100 dtn vial Ellman's Reagent (96-well kit; Item No. 400050): Reconstitute with 20 ml of UltraPure water.

   OR

   250 dtn vial Ellman's Reagent (480-well kit; Item No. 400050): Reconstitute with 50 ml of UltraPure water.

NOTE: Reconstituted Ellman's Reagent is unstable and should be used the same day it is prepared; protect the Ellman's Reagent from light when not in use. Extra vials of the reagent have been provided should a plate need to be re-developed or multiple assays run on different days.

2. Empty the wells and rinse five times with Wash Buffer.
3. Add 200 µl of Ellman's Reagent to each well.
4. Add 5 µl of tracer to the TA wells.
5. Cover the plate with a 96-Well Cover Sheet (Item No. 400012). Optimum development is obtained by using an orbital shaker equipped with a large, flat cover to allow the plate(s) to develop in the dark. This assay typically develops (*i.e.*, B_0 wells ≥0.3 A.U. (blank subtracted)) in 90-120 minutes.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.
2. Remove the plate cover being careful to keep Ellman's Reagent from splashing on the cover. *NOTE: Any loss of Ellman's Reagent will affect the absorbance readings.*
3. Read the plate at a wavelength between 405 and 420 nm.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used. The data should be plotted as either %B/B_0 *versus* log concentration using a four-parameter logistic fit or as logit B/B_0 *versus* log concentration using a linear fit. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/elisa) to obtain a free copy of this convenient data analysis tool.*

Calculations

Preparation of the Data

The following procedure is recommended for preparation of the data prior to graphical analysis.

NOTE: If the plate reader has not subtracted the absorbance readings of the blank wells from the absorbance readings of the rest of the plate, be sure to do that now.

1. Average the absorbance readings from the NSB wells.
2. Average the absorbance readings from the B_0 wells.
3. Subtract the NSB average from the B_0 average. This is the corrected B_0 or corrected maximum binding.
4. Calculate the B/B_0 (Sample or Standard Bound/Maximum Bound) for the remaining wells. To do this, subtract the average NSB absorbance from the S1 absorbance and divide by the corrected B_0 (from Step 3). Repeat for S2-S8 and all sample wells. (To obtain %B/B_0 for a logistic four-parameter fit, multiply these values by 100.)

NOTE: The TA values are not used in the standard curve calculations. Rather, they are used as a diagnostic tool; low or no absorbance froma TA well could indicate a dysfanction in the enzyme-substrate system.

Plot the Standard Curve

Plot %B/B_0 for standards S1-S8 *versus* oxytocin concentration using linear (y) and log (x) axes and perform a 4-parameter logistic fit.

Alternative Plot - The data can also be lineraized using a logit transformation. The equation for this conversion is shown below. *NOTE: Do not use %B/B_0 in this calculation.*

$$\text{logit } (B/B_0) = \ln [B/B_0/(1 - B/B_0)]$$

Plot the data as logit (B/B_0) *versus* log concentrations and perform a linear regression fit.

Determine the Sample Concentration

Calculate the B/B_0 (or %B/B_0) value for each sample. Determine the concentration of each sample using the equation obtained from the standard curve plot. *NOTE: Remember to account for any concentration or dilution of the sample prior to the addition to the well.* Samples with %B/B_0 values greater than 80% or less than 20% should be re-assayed as they generally fall out of the linear range of the standard curve. A 20% or greater disparity between the apparent concentration of two different dilutions of the same sample indicates interference which could be eliminated by purification.

Performance Characteristics

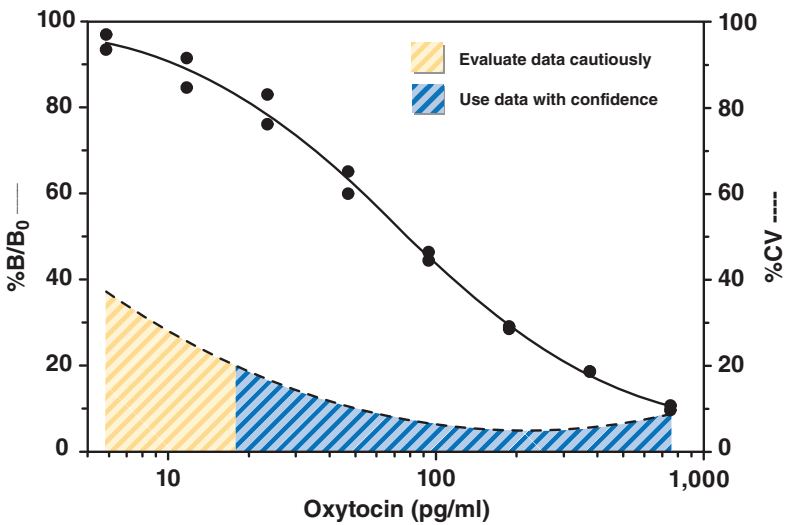
Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially.

	Raw Data		Average	Corrected
Total Activity	3.490	3.399	3.445	
NSB	0.000	0.000	0.000	
B_0	1.406	1.399		
	1.521	1.434	1.440	1.440

Dose (pg/ml)	Raw Data		Corrected		%B/B_0	
750	0.154	0.165	0.154	0.165	10.7	9.5
375	0.268	0.259	0.268	0.259	18.6	18.4
188	0.411	0.415	0.411	0.415	28.5	29.1
93.8	0.668	0.649	0.668	0.649	46.4	44.4
46.9	0.938	0.961	0.938	0.961	65.1	59.9
23.4	1.195	1.210	1.195	1.210	83.0	76.1
11.7	1.317	1.320	1.317	1.320	91.5	84.6
5.9	1.396	1.407	1.396	1.407	96.9	93.4

Table 3. Typical results



Assay Range = 5.9-750 pg/ml
Sensitivity (defined as 80% B/B_0) = 20 pg/ml
Mid-point (defined as 50% B/B_0) = 88 pg/ml
Lower Limit of Detection (LLOD) = 16 pg/ml

The sensitivity and mid-point were derived from the standard curve shown above. The standard was diluted with ELISA Buffer.

Figure 6. Typical standard curve

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 28 and in the table below.

Dose (pg/ml)	%CV* Intra-assay variation	%CV* Inter-assay variation
750	8.4	22.0
375	5.8	12.4
188	6.3	10.7
93.8	7.2	7.0
46.9	9.6	6.3
23.4	15.8	14.6
11.7	25.7**	23.9**
5.9	37.7**	22.6**

Table 4. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
**Evaluate data in this range with caution

Cross Reactivity:

Compound	Cross Reactivity
Oxytocin	100%
Mesotocin	100%
Isotocin	100%
Arg-Vasopressin	<0.01%
Arg-Vasotocin	<0.01%
Dynorphin A	<0.01%
Lys-Vasopressin	<0.01%
Vasoactive Intestinal Peptide (VIP)	<0.01%
Tocinoic Acid	<0.01%
Melanostatin	<0.01%
MIF-1	<0.01%

Table 5. Cross Reactivity of the Oxytocin ELISA

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
High NSB (>10% of B_0)	A. Poor washing B. Exposure of NSB wells to specific antibody	A. Rewash plate and redevelop
Very low B_0	A. Trace organic contaminants in the water source B. Plate requires additional development time C. Dilution error in preparing reagents	A. Replace activated carbon filter or change source of UltraPure water B. Return plate to shaker and re-read later
Low sensitivity (shift in dose response curve)	Standard is degraded	Replace standard
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present	Purify sample prior to analysis by ELISA[9]
Only Total Activity (TA) wells develop	Trace organic contaminants in the water source	Replace activated carbon filter or change source of UltraPure water

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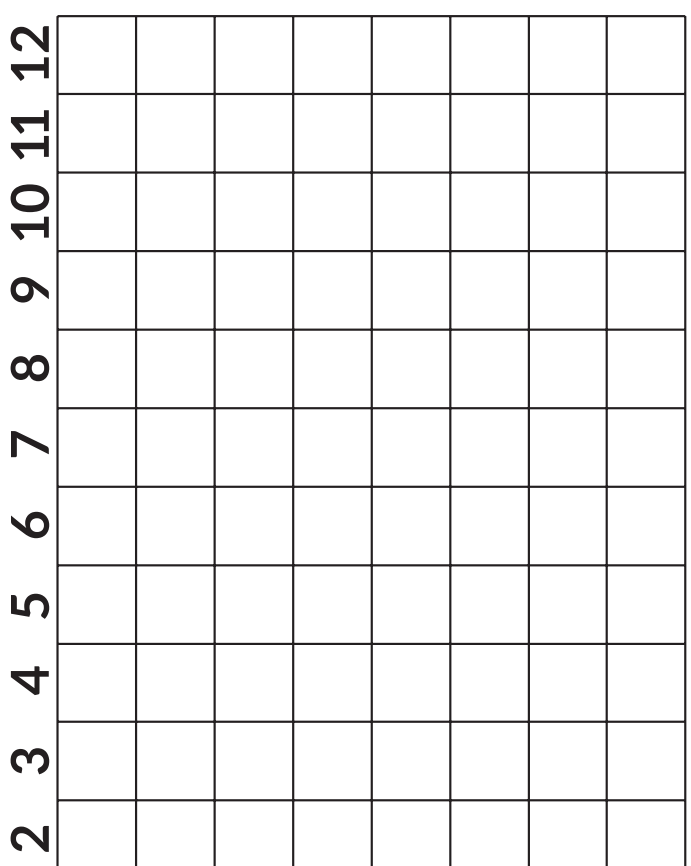

NOTES

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