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ab133050
Oxytocin ELISA Kit

For quantitative detection of Oxytocin in Tissue Culture media, Cell Culture Supernatant, Human Serum, Saliva, Plasma, Milk, Cerebrospinal Fluid and Urine.

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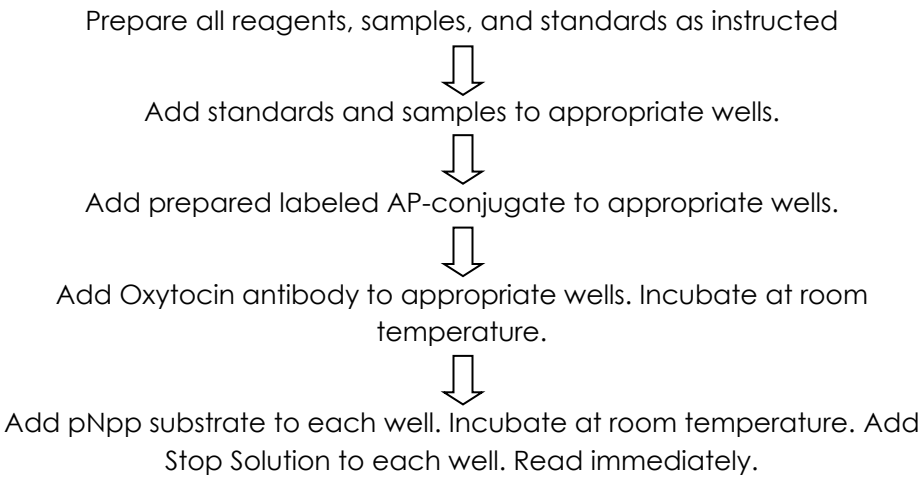
1. Overview

Abcam's Oxytocin *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Oxytocin in Tissue culture media, Cell culture supernatant, Human Serum, Saliva, Plasma, Milk, Cerebrospinal Fluid and Urine.

A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-Oxytocin antigen and a polyclonal rabbit antibody specific to Oxytocin. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of Oxytocin captured in the plate.

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 disulfide bond and a semi-flexible carboxyamidated tail. A hormone once thought to be limited to female smooth muscle reproductive physiology, more current findings have determined that oxytocin also functions as a neurotransmitter, may be involved in neuropsychiatric disorders, social/sexual behavior and is important in male reproductive physiology. Oxytocin and the related neurohypophysial peptide, Arg 8 -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, amphibians, marsupials and nonmammalian 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 intracellular response cascade via a phosphoinositide signaling pathway.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt, apart from the Alkaline Phosphatase Conjugate and Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 wells	+4°C
Oxytocin Alkaline Phosphatase Conjugate	5 mL	-20°C
Oxytocin Antibody	5 mL	+4°C
Oxytocin Standard	500 µL	-20°C
Assay Buffer	27 mL	+4°C
20X Wash Buffer Concentrate	27 mL	+4°C
pNpp Substrate	20 mL	+4°C
Stop Solution	5 mL	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- Eppendorf tubes.
- Microplate Shaker.
- Absorbent paper for blotting.
- 200 mg C18 Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of Oxytocin).
- Deionized water.
- Acetonitrile, anhydrous (>99%).
- Trifluoroacetic acid (>99%).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Standards can be made up in either glass or plastic tubes.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent.
- Pipette standards and samples to the bottom of the wells.
- Add the reagents to the side of the well to avoid contamination.
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results.
- The Oxytocin Standard provided, is supplied in ethanolic buffer at a pH optimized to maintain Oxytocin integrity. Care should be taken handling this material because of the known and unknown effects of Oxytocin.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 Oxytocin Conjugate

Allow the Oxytocin Alkaline Phosphatase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare serially diluted standards immediately prior to use.
- Diluted standards should be used within 60 minutes of preparation.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Standard Reconstitution:

10.1.1 For urine/saliva/serum/milk/plasma/cerebrospinal fluid samples: reconstitute the Oxytocin standard by diluting standards with Assay Buffer.

10.1.2 For tissue culture media and cell culture supernatant samples: reconstitute the Oxytocin standard by diluting standards with tissue culture media.

10.2 Allow the 10,000 pg/mL Oxytocin Standard solution to equilibrate to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.3 Label 7 tubes #1 – #7.

10.4 Prepare a 1,000 pg/mL **Standard 1** by adding 100 µL of the 10,000 pg/mL to Stock Standard to 900 µL of the appropriate sample diluent (as per 10.1.1 or 10.1.2) into tube #1. Mix thoroughly and gently.

10.5 Add 500 µL of the appropriate sample diluent into tube #2 – #7.

10.6 Prepare **Standard 2** by transferring 500 µL from Standard 1 to tube #2. Mix thoroughly and gently.

10.7 Prepare **Standard 3** by transferring 500 µL from Standard 2 to tube #3. Mix thoroughly and gently.

10.8 Using the table below as a guide, repeat for tubes #4 through #7.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	100 µL Standard	900	10,000	1,000
2	500 µL Standard #1	500	1,000	500
3	500 µL Standard #2	500	500	250
4	500 µL Standard #3	500	250	125
5	500 µL Standard #4	500	125	62.5
6	500 µL Standard #5	500	62.5	31.2
7	500 µL Standard #6	500	31.2	15.6

11. Sample Preparation

- The Oxytocin ELISA is compatible with samples from a number of matrices. Oxytocin samples diluted sufficiently into the kit Assay Buffer can be read directly from the standard curve
- Samples in the majority of tissue culture media, including those containing fetal bovine serum, can also be read in the assay, provided the standards have been diluted into the tissue culture media instead of Assay Buffer
- There will be a small change in binding associated with running the standards and samples in media. The user must verify that the recommended dilutions are appropriate for their samples
- Samples containing rabbit IgG may interfere with the assay
- Because of the labile nature of oxytocin we recommend several precautions in collecting and analyzing samples. Blood samples should be drawn into chilled serum or EDTA (1 mg/mL blood) tubes containing Aprotinin (500 KIU/mL of blood). Centrifuge the samples at 1600 x g for 15 minutes at 4°C. Transfer the plasma or serum to a plastic tube and store at -70°C or lower for long term storage. Avoid repeated freeze/thaw cycles. A suitable extraction procedure is outlined below:

11.1 Add an equal volume of 0.1% trifluoroacetic acid (TFA) in water (TFA-H_2O) to the sample. Centrifuge at 17000g for 15 minutes at 4°C to clarify and save the supernatant.

11.2 Equilibrate a 200mg C18 Sep-Pak column with 1ml of acetonitrile, followed by 10-25mL of 0.1% TFA-H_2O.

11.3 Apply the supernatant to the Sep-Pak column and wash with 10-20mL of 0.1% TFA-H_2O. Discard wash.

11.4 Elute the sample slowly (gravity-fed) by applying 3mL of a solution comprised of 95% acetonitrile/5% of 0.1% TFA-H_2O. Collect the eluate in a plastic tube.

11.5 Evaporate to dryness under argon or nitrogen gas or with the aid of a centrifugal concentrator under vacuum. Evaporation under cold temperature is recommended. Store at -20°C.

11.6 Reconstitute with Assay Buffer and measure immediately. You will need to have at least 250µL volume (upon reconstitution) per sample in order to have enough material to run duplicates (n=2 per sample). Please note that upon reconstitution insoluble material may be observed in some samples. Care should be taken to avoid this material when adding sample to plate wells. Please note that recovery of peptides from extraction processes

can be variable. It is important to optimize any process to obtain optimum recoveries. Extraction efficiencies can be determined by a number of methods, including the use of radioactive peptide, or by spiking into paired samples and determining the recovery of this known amount of added Oxytocin.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

Recommended plate layout

	1	2	3	4
A	B_s	Std 1	Std 5	Sample 2
B	B_s	Std 1	Std 5	Sample 2
C	TA	Std 2	Std 6	etc
D	TA	Std 2	Std 6	etc
E	NSB	Std 3	Std 7	
F	NSB	Std 3	Std 7	
G	B_0	Std 4	Sample 1	
H	B_0	Std 4	Sample 1	

Key:

B_s = Blank; contains substrate only.

TA = Total Activity; contains conjugate (5 µL) and substrate.

NSB = Non-specific binding; contains standard diluent, assay buffer, conjugate and substrate.

B_0 = 0 pg/mL standard; contains standard diluent, conjugate, antibody and substrate.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Refer to the recommended plate layout in Section 12 before proceeding with the assay

13.1 Add 100 µL of appropriate diluent* into the NSB (non-specific binding) wells. (*Use the same diluent used to prepare standards in section 10, either Assay Buffer or tissue culture media).

13.2 Add 100 µL appropriate diluent (Assay Buffer or tissue culture media) into the B_0 (0 pg/mL standard) wells.

13.3 Add 100 µL of prepared standards and 100 µL diluted samples to appropriate wells.

13.4 Add 50 µL of Assay Buffer into NSB wells.

13.5 Add 50 µL of the Oxytocin-alkaline phosphatase conjugate (blue) into NSB, B_0, standard and sample wells, i.e. not TA (Total Activity) and B_s (blank) wells.

13.6 Add 50 µL of Oxytocin antibody (yellow) into B_0, standard and sample wells, i.e. not B_s, TA or NSB wells.

13.7 *Note:* Every well used should be green except the NSB wells which should be blue. The B_s and TA wells are empty at this point and have no color.

13.8 Tap the plate gently to mix. Seal the plate and incubate at 4°C for 18-24 hours.

13.9 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.10 Add 5 µL of the Oxytocin-alkaline phosphatase conjugate to the TA wells.

13.11 Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.

13.12 Add 50 µL Stop Solution to all wells.

13.13 After blanking the plate reader against the B_s (blank) wells, read optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked

against the B_s wells, manually subtract the mean optical density of the blank wells from all readings.

14. Calculations

14.1 Calculate the average net absorbance measurement (Average Net OD) for each standard and sample by subtracting the average NSB absorbance measurement from the average absorbance measurement (Average OD) for each standard and sample.

Average Net OD = Average Bound OD - Average NSB OD

14.2 Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (B_0), using the following formula:

Percent Bound = (Average Net OD/Average Net B_0 OD) x 100

14.3 Plot the Percent Bound (B/B_0) and the net OD versus concentration of aldosterone for the standards. The concentration of aldosterone in the unknowns can be determined by interpolation.

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.

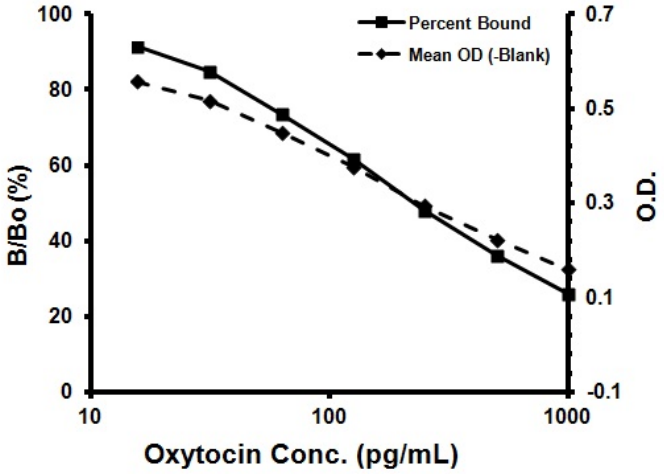


Figure 1. Example of Oxytocin standard curve.

Sample	Mean OD (-Blank)	% Bound	Oxytocin pg/mL
B_s	(0.073)	-	-
TA	0.803	-	-
NSB	0.002	0	-
Standard 1	0.160	26.0	1,000
Standard 2	0.222	36.2	500
Standard 3	0.294	48.1	250
Standard 4	0.377	61.8	125
Standard 5	0.449	73.6	62.5
Standard 6	0.517	84.8	31.2
Standard 7	0.557	91.4	15.6
Bo	0.609	100	0
Unknown1	0.244	39.9	397
Unknown 2	0.359	58.8	145

Typical Quality Control Parameters

Total Activity Added = 0.803 x 10 = 8.03

%NSB = 0.0%

%Bo/TA = 7.56%

Quality of Fit = 1.0000 (Calculated from 4 parameter logistic curve fit)

20% Intercept = 1,791 pg/mL

50% Intercept = 228 pg/mL

80% Intercept = 43 pg/mL

16. Typical Sample Values

SENSITIVITY –

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard 7. The detection limit was determined as the concentration of oxytocin measured at two (2) standard deviations from the zero along the standard curve was determined to be 15.0 pg/mL.

SAMPLE RECOVERY –

Recovery was determined by Oxytocin in tissue culture media, Human saliva, serum, cerebrospinal fluid, and urine. Mean recoveries are as follows:

Sample Type	Average % Recovery	Recommended Dilution
Tissue Culture Media	92	Neat
Human Saliva	90	1:32
Human Urine	111	1:16
Human Serum	104	1:8
Human Cerebrospinal Fluid	106	Neat

LINEARITY OF DILUTION –

A serum sample spiked with 200pg/mL Oxytocin was extracted then reconstituted at the same volume with assay buffer. Next the sample was serially diluted 1:2 with Assay Buffer and measured in the assay. The recovered concentration was determined to be linear within a range of 100% ± 15% relative to a designated dilution.

Dilution Factor	Recovered Spike Concentration (pg/mL)	Dilutional Linearity
1	233.1	91
2	275.0	104
4	262.8	100
8	283.9	106

Intra-Assay

Determined by assaying 20 replicates of 3 controls containing oxytocin in a single assay.

	Oxytocin (pg/mL)	%CV
Low	39.9	12.6
Medium	121.4	10.2
High	363.7	13.3

Inter-Assay

Determined by assaying controls of varying oxytocin concentration in multiple assays (n = 17) over several days.

	Oxytocin (pg/mL)	%CV
Low	47.0	20.9
Medium	145.1	16.5
High	397.2	11.8

17. Assay Specificity

CROSS REACTIVITY –

Compound	Cross Reactivity (%)
Mesotocin	7.0
Arg^8 -Vasotocin	7.5
Ser^4 ,Ile^8 -Oxytocin	<0.02
TRH	<0.02
Growth Hormone	<0.02
Tocinoic acid	<0.02
Melanostatin	<0.02
Somatostatin	<0.02
Met-Enkephalin	<0.02
VIP	<0.02
Lys^8 – Vasopressin	<0.02
Arg^8 - Vasopressin	<0.02
a -ANP	<0.02

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

Technical Support

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