Version 26-02/25

User's Manual

Oxytocin Enzyme Immunoassay Kit

REF DEIABL252

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics
Address: 45-1 Ramsey Road, Shirley, NY 11967, USA
Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) Fax: 1-631-938-8221
Email: info@creative-diagnostics.com Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

The Oxytocin Enzyme Immunoassay Kit is designed to quantitatively measure Oxytocin present in serum, plasma, saliva, clarified milk and tissue culture media samples.

General Description

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 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.

Principles of Testing

An oxytocin standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture rabbit antibodies. An oxytocin-peroxidase conjugate is added to the standards 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°C the plate is washed and supplied substrate is added. The substrate reacts with the bound oxytocin-peroxidase conjugate. After a 30 minute incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. 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.

Reagents And Materials Provided

1. Coated Clear 96 Well Plates

Clear plastic microtiter plate(s) with break-apart strips coated with goat anti-rabbit IgG. 1 Each

2. Oxytocin Standard

Oxytocin at 100,000 pg/mL in a special stabilizing solution. 125 µL

3. Oxytocin Antibody

A rabbit polyclonal antibody specific for oxytocin. 3 mL

4. Oxytocin Conjugate

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

5. Assay Buffer Concentrate

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

6. Extraction Solution

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

7. Wash Buffer Concentrate

A 20x concentrate that should be diluted with deionized or distilled water. 30 mL

8. TMB Substrate

11 mL

9. Stop Solution

A 1M solution of hydrochloric acid. CAUSTIC. 5 mL

10. Plate Sealer

1 Each

Materials Required But Not Supplied

1. Distilled or deionized water.
2. A Speedvac/centrifugal concentrator or N2 gas and gas manifold for evaporation.
3. Repeater pipet, such as an Eppendorf repeater, with disposable tips to accurately dispense 25 µL, 50 µL, and 100 µL.
4. A microplate shaker.
5. Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.
6. Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Storage

4°C

Specimen Collection And Preparation

SAMPLE TYPES

This assay has been validated for serum, EDTA and heparin plasma, clarified 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 isotocin and mesotocin this kit should also be able to measure mesotocin from birds, fish, amphibians, and reptiles. The end user should evaluate recoveries of oxytocin in other samples being

tested.

SAMPLE PREPARATION

Serum and Plasma Samples

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:

1. Mix 1 part sample with 1.5 parts of Extraction Solution.
2. Vortex and then nutate at room temperature for 90 minutes.
3. Centrifuge for 20 minutes at 4°C at 1660 x g.
4. Speedvac supernatant to dryness at 37°C.
5. Reconstitute sample with 250 µL of Assay Buffer

Saliva Samples

Saliva samples should be extracted using the extraction reagent as described for serum and plasma samples. Saliva should be collected with Salivettes, extracted, dried, and reconstituted in 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.

Reconstitution And Storage

This kit should be stored at 4°C until the expiration date of the kit.

Reagent Preparation

Allow the kit reagents to come to room temperature for 30 minutes. 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.

Standard 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 10,000, 4,000, 1,600, 640, 256, 102.4, 40.96 and 16.38 pg/mL.

Use all Standards 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)	10,000	4,000	1,600	640	256	102.4	40.96	16.38

Assay Procedure

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine oxytocin concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard 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 standards into wells in the plate.
3. Pipet 100 µL of Assay Buffer into the maximum binding (B0 or Zero standard) wells.
4. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
5. Add 25 µL of Oxytocin Conjugate to each well using a repeater pipet.
6. Add 25 µL of 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°C for 16-18 hours.
8. The following day, remove the TMB Substrate from the refrigerator and allow to come to room temperature for at least 30 minutes. Addition of cold Substrate will cause depressed signal.
9. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
10. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
11. Incubate the plate at room temperature for 30 minutes without shaking.
12. Add 50 µL of the Stop Solution to each well, using a repeater or a multichannel pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. Use the plate reader's built-in 4PLC software capabilities to calculate oxytocin concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

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 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.

Typical Standard Curve

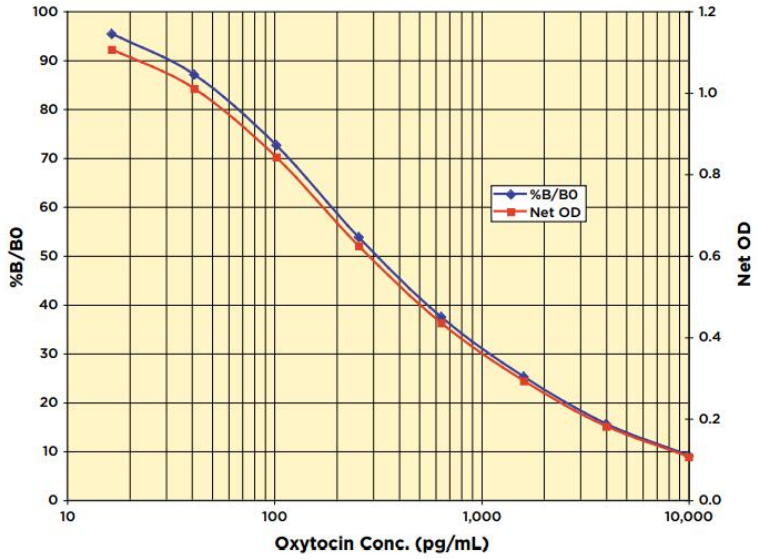
TYPICAL DATA

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.

Typical Standard Curves:

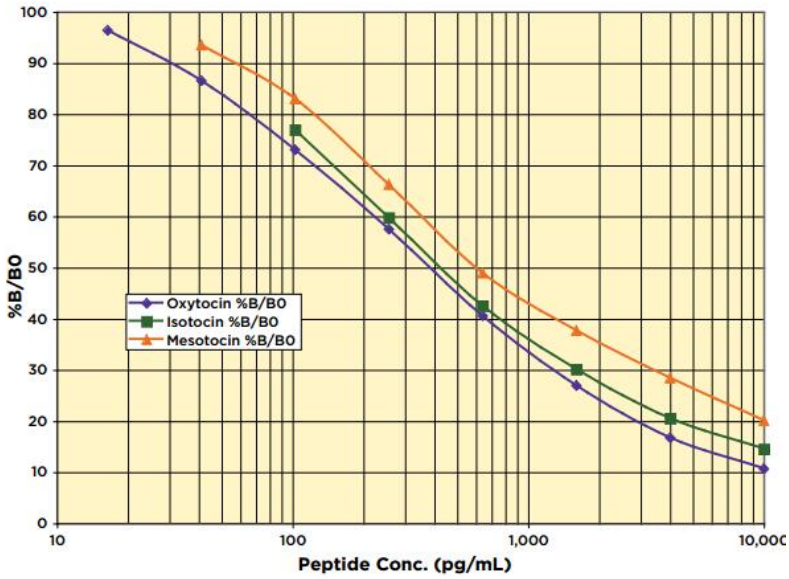


Always run your own standard curves for calculation of results. Do not use this data.

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.



Performance Characteristics

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%

Precision

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%

Detection Limit

22.9 pg/mL

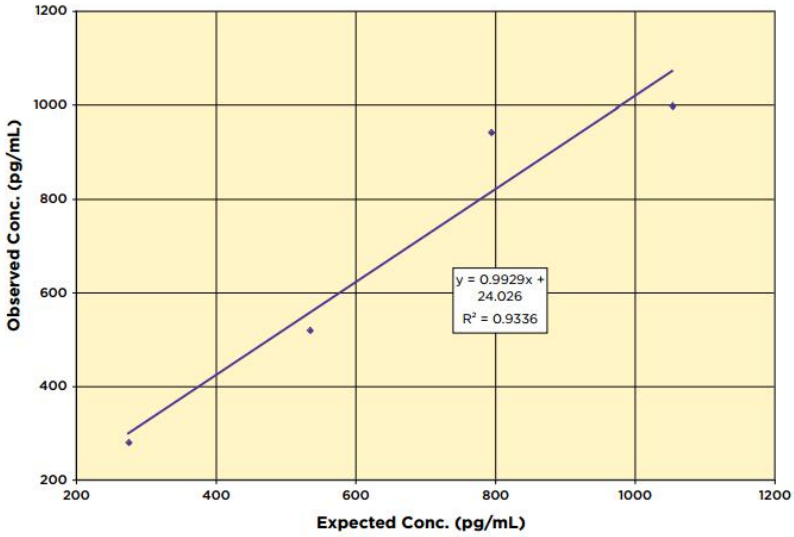
Sensitivity

17.0 pg/mL

Linearity

Linearity was determined by taking two diluted samples, one with a low level of 16.3 pg/mL and one with a higher level of oxytocin 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%**



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 as prepared on the protocol.

Limitations

CD Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose. 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.

References

1. G.L. Kovacs, & D.H.K. Versteeg, "Neurohypophysial Peptides and Brain Neurochemistry." 1993, Ann NY Acad. Sci., 689:7 309-319.
2. Insel TR, Young L, and Wang Z, "Central oxytocin and reproductive behaviours." Rev. Reprod., 1997, 2(1): 28-37.
3. A. Frasch, et al., "Oxytocin: Cellular and Molecular Approaches", 1995, NY: Plenum Press.
4. M.M. McCarthy, & M. Altemus, "Central nervous system actions of oxytocin and modulation of behavior in humans.", Mol. Med. Today, 1997, 3(6): 269-275.
5. A. Argiolas, & M.R. Melis, "Oxytocin: Cellular and Molecular Approaches", 1995, NY: Plenum Press.
6. K.P. Conrad, et al., "Influence of oxytocin on renal hemodynamics and sodium excretion.", Ann. NY Acad. Sci., 1993, 689: 346-362.
7. R. Archer, & J. Chauvet, "The neurohypophysial endocrine regulatory cascade: precursors, mediators, receptors, and effectors.", Front. Neuroendo., 1995, 16: 237-289.
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.