



**Oxytocin
ELISA kit
(96 Tests)**

Zellbio GmbH (Germany)

CAT No. ZX-55106-96

www.zellx.de

Sample Types Validated for:

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

!!! Caution: This product is for Research Use Only. Not for *in vitro* Diagnostics !!!

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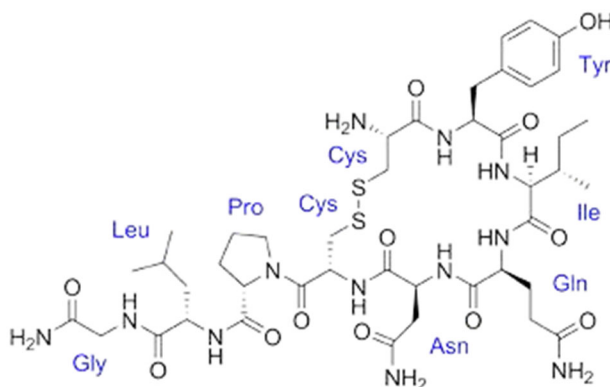
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Please read this insert completely prior to using the product.

Introduction

Background

Oxytocin is a neurohypophysial peptide consisting of nine amino acids with a disulfide bond between residues 1 and 6 and a semi-flexible carboxyamidedated tail. It is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior pituitary. It was initially thought that this hormone is limited to female smooth muscle reproductive physiology, however, recent studies have demonstrated the role of Oxytocin as a neurotransmitter in various behaviors including orgasm, social recognition, pair bonding, anxiety, and maternal behaviors, as well as in male reproductive physiology. Additionally, Oxytocin and the related neurohypophysial peptide, Arg⁸- Vasopressin, maintain renal water and sodium balance. 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.



Assay principle

The ZellX® Oxytocin Immunoassay kit is a competitive ELISA assay to quantitatively measure Oxytocin present in serum, plasma, saliva, clarified milk, and tissue culture media samples. An Oxytocin stock solution is provided to generate a standard curve for the assay and all samples should be read off the standard curve.

The kit includes a 96-well plate that is pre-coated with a secondary anti-rabbit antibody. The function of this antibody is to capture the rabbit anti-Oxytocin antibody bound to Oxytocin conjugate (peroxidase-labeled) and hold this complex to the plate during the subsequent detection steps. The Oxytocin-conjugate (labeled) and the sample Oxytocin (unlabeled) compete for binding to the rabbit antibody. After overnight incubation at 4°C, the substrate is added to react with the peroxidase-labeled antibody-antigen conjugate. After stopping the reaction, the intensity of the generated color can be measured at 450 nm. The lower the amount of Oxytocin in the sample, the stronger the signal due to more labeled Oxytocin bound to the well.

This kit uses Oxytocin Standard solutions calibrated to the 4th WHO International Standard NIBSC.

General information

Materials supplied in the Kit

Component	Quantity
Oxytocin Standard (100 ng/mL)	125 µL
Oxytocin Antibody	2.6 mL
Oxytocin Conjugate	2.6 mL
Assay Buffer Concentrate (5x)	11 mL
Wash Buffer Concentrate (20x)	25 mL
Extraction Solution	50 mL
TMB Substrate	11 mL
Stop Solution	5 mL
Coated Clear 96-Well Plate & Sealer	1 plate

Storage instruction

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

Materials required but not supplied

Deionized water (diH₂O)

Microplate/ELISA reader capable of reading optical absorption at 450 nm

Centrifuge, Vortex mixer, microplate shaker

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Polypropylene or glass test tubes

A Speedvac/centrifugal concentrator or N₂ gas and gas manifold for evaporation

Precautions

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.

Stop Solution is an acidic solution and should not come in contact with skin or eyes. Handling this reagent needs appropriate precaution.

General remarks

- Equilibrate all kit components to room temperature (RT) 30 minutes before use.
- The instruction must be strictly followed.
- The reading of Microplate/ELISA reader must be set at the appropriate wavelength.
- Pipette tips should not be used more than once in order to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- 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 Wash Buffers from other manufacturers, containing sodium azide will inhibit color production by 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.

Assay protocol

Reagent preparation

- i. **Assay Buffer:** Prepare a 1:5 dilution of Assay Buffer Concentrate with diH₂O (1 part Assay Buffer Conc. with 4 parts diH₂O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.
- ii. **Wash Buffer:** Prepare a 1:20 dilution of Wash Buffer Concentrate with diH₂O (1 part Wash Buffer Conc. with 19 parts diH₂O), and mix well. Assay Buffer can be stored at room temperature for up to 3 months.

Sample preparation

Since Oxytocin is identical across all species, it is expected that this kit can measure Oxytocin in human and other species. Due to cross reactivity to Isotocin and Mesotocin, this kit should also be able to measure Mesotocin from birds, fish, amphibians, and reptiles. Recoveries of Oxytocin in other samples should be evaluated by end user.

Samples containing visible particulate should be centrifuged prior to conducting the assay. Moderate to severely hemolyzed samples should not be used for this assay.

Extraction solution must be used only with Serum and Plasma samples.

All samples and standards must be used within 2 hours of preparation.

I. **Serum, Plasma:**

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

II. **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.

III. **Milk:**

- Centrifuge at 10000 g for 15 minutes. Pierce the top fatty layer and collect the liquid supernatant.
- Repeat the centrifugation and liquid collection two more times.
- The final collected supernatant liquid must then be diluted $\geq 1:10$ with the provided Assay Buffer prior to performing assay.
- The clarified milk sample can be stored at -20°C for later use.

IV. **Tissue Culture Media:**

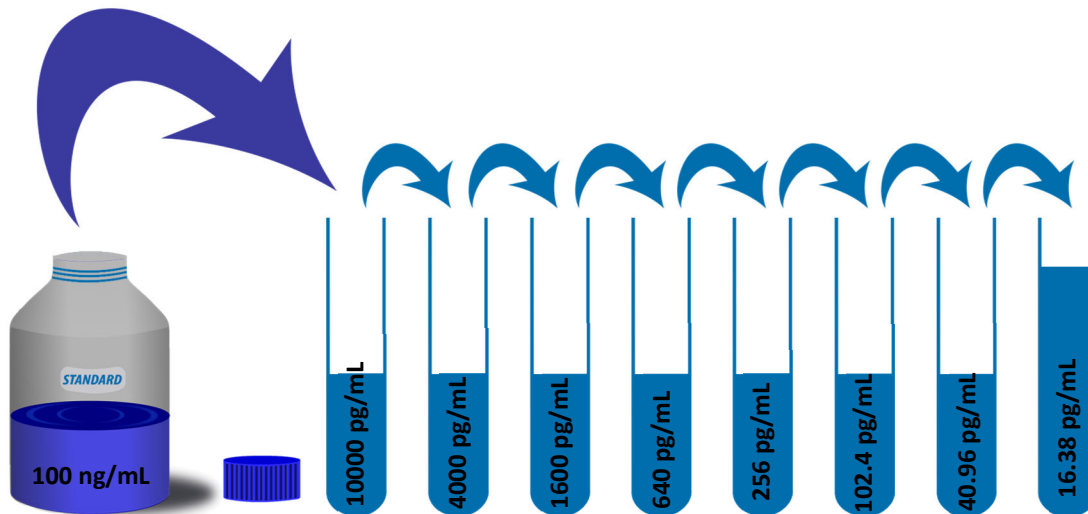
- For measuring Oxytocin in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM.

All the samples must be used within 2 hours of preparation.

Standard preparation

- Prepare a 1:10 dilution of Oxytocin Standard with Assay Buffer (mix 50 μL of standard with 450 μL of Assay Buffer), and label as the Standard No.8 (10000 pg/mL).
- Make series of lower dilutions as described in the table.
- The Assay Buffer is used as the 0 pg/mL standard.

No.	Concentration	Material needed
Standard No.8	10000 pg/mL	50 μL Oxytocin Standard + 450 μL Assay Buffer
Standard No.7	4000 pg/mL	200 μL Standard No.8 + 300 μL Assay Buffer
Standard No.6	1600 pg/mL	200 μL Standard No.7 + 300 μL Assay Buffer
Standard No.5	640 pg/mL	200 μL Standard No.6 + 300 μL Assay Buffer
Standard No.4	256 pg/mL	200 μL Standard No.5 + 300 μL Assay Buffer
Standard No.3	102.4 pg/mL	200 μL Standard No.4 + 300 μL Assay Buffer
Standard No.2	40.96 pg/mL	200 μL Standard No.3 + 300 μL Assay Buffer
Standard No.1	16.38 pg/mL	200 μL Standard No.2 + 300 μL Assay Buffer
Standard No.0	0 pg/mL	200 μL Assay Buffer



All standard must be used within 2 hours of preparation

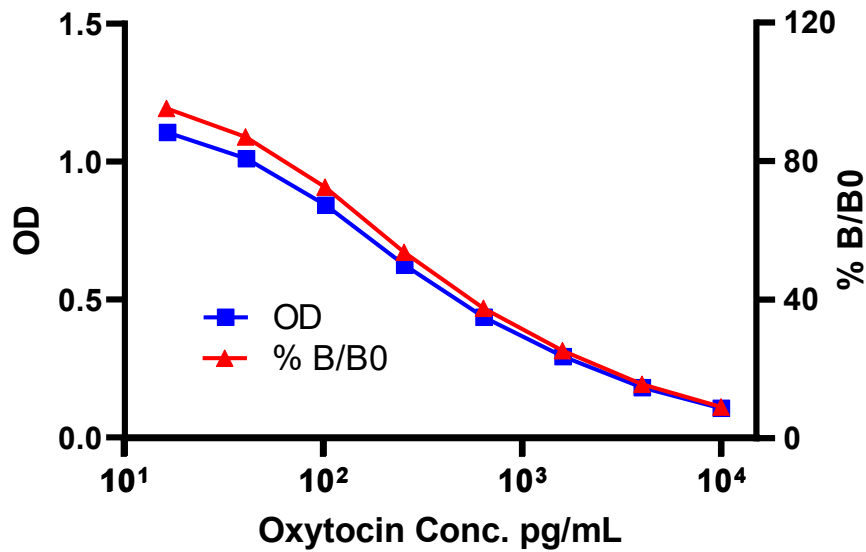
Assay Procedure

1. Pipette 100 μL of either samples or standards into duplicate wells in the plate.
2. Pipette 100 μL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 125 μL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 μL of Oxytocin Conjugate to each well, using a repeater pipette.
5. Add 25 μL of Oxytocin Antibody to each well, except the NSB wells, using a repeater pipette.
6. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
7. Shake the plate in a plate shaker at room temperature for 15 minutes.
8. Cover the plate with the plate sealer and incubate for 16-18 hour at 4°C.
9. The following day, remove the TMB Substrate from the refrigerator and allow to Equilibrate to room temperature for at least 30 minutes. Addition of cold TMB Substrate will cause depressed signal.
10. Aspirate the plate and wash each well 4 times with 300 μL Wash Buffer.
11. Tap the plate on clean absorbent towels to dry.
12. Add 100 μL of TMB Substrate to each well using a multichannel/repeater pipette.
13. Incubate at room temperature for 30 minutes without shaking.
14. Add 50 μL of Stop Solution to each well using a multichannel/repeater pipette.
15. Read the optical density at 450 nm.

Calculation

- Average the duplicate optical density (OD) readings for each standard and sample.
- Subtract the mean ODs of the NSB from all OD values
- Create a standard curve by reducing the data using the four parameter logistic curve (4PLC) fitting routine on the plate reader
- Calculate the % B/B0 ratio.
 - **Note:** B0 is the binding for the zero standard or the maximum binding well, which represents the maximum signal from enzyme captured by the specific antibody in competitive ELISA. All other standards and samples are expressed as a percentage of this value (% B/B0).
- The concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Conversion Factor: 100 pg/mL of Oxytocin is equivalent to 99.3 pM



A typical standard curve of ZellX[®] Oxytocin Assay kit

Run your own standard curves for calculation of results

Assay range

The detection limit of ZellX[®] Oxytocin assay was determined as 22.9 pg/mL.

Sensitivity

The sensitivity of the ZellX[®] Oxytocin assay was determined as 17.0 pg/mL.

Precision

Intra-Assay Precision (Precision within an assay): 2 human serum samples were tested 20 times in an assay.

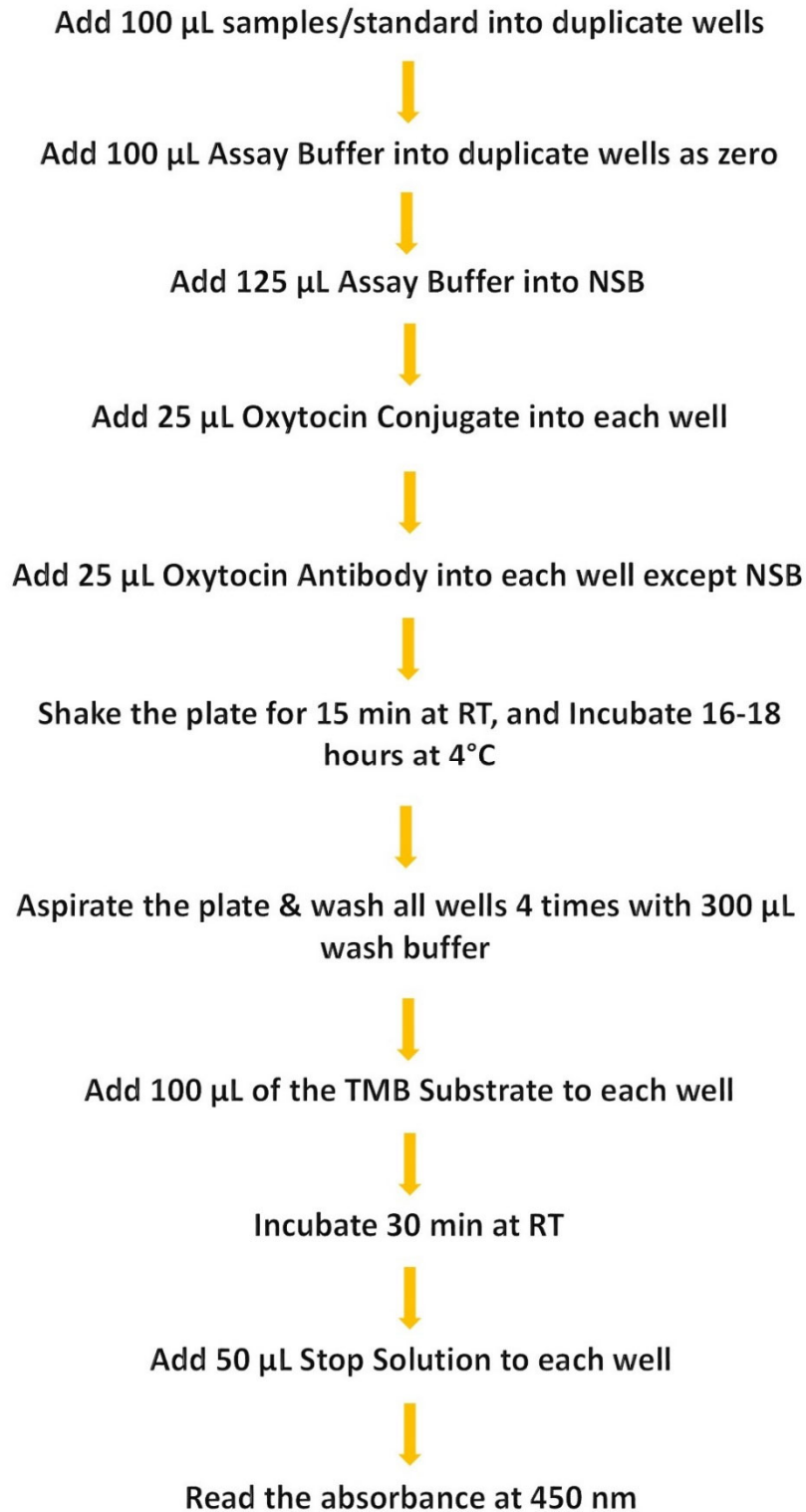
Inter-Assay Precision (Precision between assays): 2 human serum samples were tested in duplicate on 17 different assays over multiple days.

<i>Item</i>	<i>%CV</i>
Intra assay	5.2, 4.3
Inter assay	10.0, 7.7

Cross Reactivity

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

<i>Steroid</i>	<i>Cross Reactivity (%)</i>
Oxytocin	100
Isotocin	94.30
Mesotocin	88.40
Lys⁸-Vasopressin	0.14
Arg⁸-Vasotocin	0.13
Arg⁸-Vasopressin	0.12

Protocol summary

References

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