Oxytocin

CLIA kit

(96 Tests)

Zellbio GmbH (Germany)

CAT No. ZX-66106-96

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 !!!

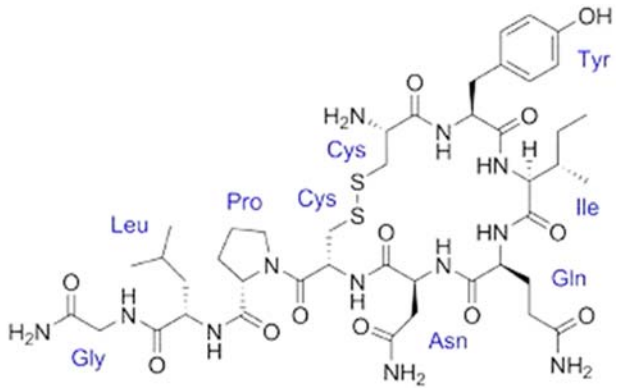
Table of Contents

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 bound between residues 1 and 6 and a semi-flexible carboxyamidated 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, Arg^8- 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 Chemiluminescent 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 chemiluminescent substrate is added to react with the peroxidase-labeled antibody-antigen conjugate to produce light. The generated light can be measured in a microtiter plate reader capable of reading luminescence. 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 (50 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
Substrate A	5.6 mL
Substrate B	5.6 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 (diH_2O)

Centrifuge, Vortex mixer, microplate shaker

Precision pipettes, multichannel/repeater pipettes and disposable pipette tips

Polypropylene or glass test tubes

A Speedvac/centrifugal concentrator or N_2 gas and gas manifold for evaporation

Microplate reader capable of reading glow chemiluminescence.

Note: All luminometers read Relative Light Units (RLU). These RLU readings will vary with brand or model of plate reader. The number of RLUs obtained depends on the sensitivity and gain of the reader used. If you are not sure how to properly configure your reader, contact your plate reader manufacturer or carry out the following protocol:

Dilute 5 µL of the Conjugate Working Solution into 45 µL of deionized water. Pipet 5 µL of diluted conjugate into a white well and add 100 µL of prepared CLIA substrate. This well will give an intensity 2-3

times the maximum binding for the assay. Adjust the gain or sensitivity so that your reader is giving close to the maximum signal.

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.

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 diH_2O (1 part Assay Buffer Conc. with 4 parts diH_2O), 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 diH_2O (1 part Wash Buffer Conc. with 19 parts diH_2O), and mix well. Assay Buffer can be stored at 4°C for up to 3 months.

iii. **Chemiluminescent Substrate:** Mix one part of the Substrate A with one part of Substrate B in a brown bottle. Chemiluminescent Substrate can be stored at 4°C for up to 1 month.

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.

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 ≥ 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:**

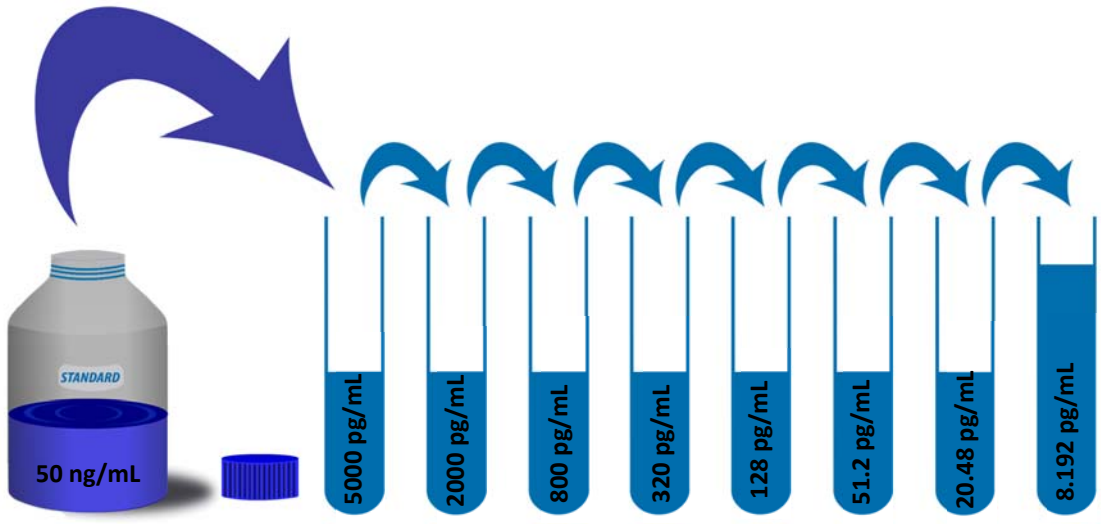
- For measuring Oxytocin in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM.

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 (5000 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	5000 pg/mL	50 µL Oxytocin Standard + 450 µL Assay Buffer
Standard No.7	2000 pg/mL	200 µL Standard No.8 + 300 µL Assay Buffer
Standard No.6	800 pg/mL	200 µL Standard No.7 + 300 µL Assay Buffer
Standard No.5	320 pg/mL	200 µL Standard No.6 + 300 µL Assay Buffer
Standard No.4	128 pg/mL	200 µL Standard No.5 + 300 µL Assay Buffer
Standard No.3	51.2 pg/mL	200 µL Standard No.4 + 300 µL Assay Buffer
Standard No.2	20.48 pg/mL	200 µL Standard No.3 + 300 µL Assay Buffer
Standard No.1	8.192 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

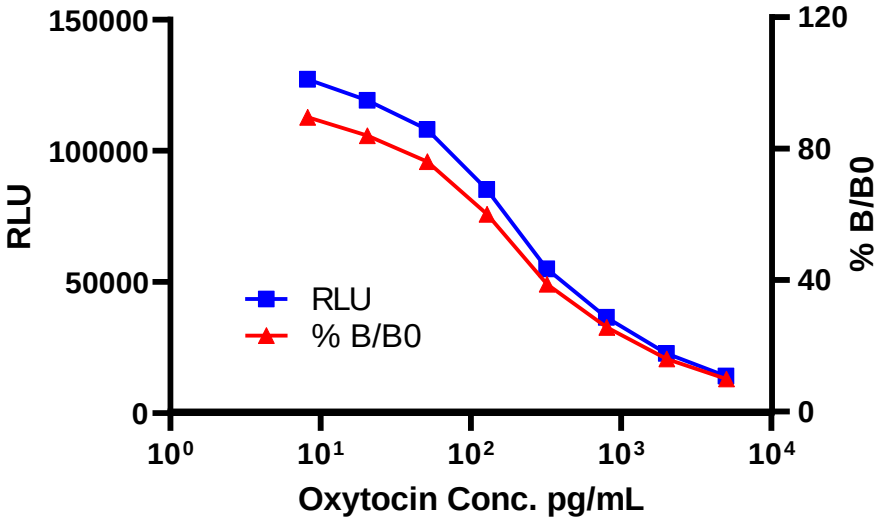
1. Pipette 100 µL of either samples or standards into duplicate wells in the plate.
2. Pipette 100 µL of Assay Buffer into duplicate wells of the Zero standard.
3. Pipette 125 µL of Assay Buffer into duplicate wells of the nonspecific binding (NSB).
4. Add 25 µL of Oxytocin Conjugate to each well, using a repeater pipette.
5. Add 25 µL of Oxytocin Antibody to each well, except the NSB wells, using a repeater pipette.
6. Shake the plate in a plate shaker at room temperature for 15 minutes.
7. Cover the plate with the plate sealer and incubate for 16 hour at 4°C.
8. The following day, remove the Chemiluminescent Substrate from the refrigerator and allow to Equilibrate to room temperature for at least 30 minutes. Addition of cold Chemiluminescent Substrate will cause depressed signal.
9. Aspirate the plate and wash each well 4 times with 300 µL Wash Buffer.
10. Tap the plate on clean absorbent towels to dry.
11. Add 100 µL of Chemiluminescent Substrate to each well using a multichannel/repeater pipette.
12. Incubate at room temperature for 5 minutes without shaking.
13. Read the luminescence generated from each well in a multimode or chemiluminescent plate reader using a 0.1 second read time per well.

➢ The chemiluminescent signal will decrease about 40% over 60 minutes.

Calculation

- Average the duplicate RLU readings for each standard and sample.
- Subtract the mean RLUs 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. 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 15.5 pg/mL.

Sensitivity

The sensitivity of the ZellX® Oxytocin assay was determined as 6.33 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 14 different assays over multiple days.

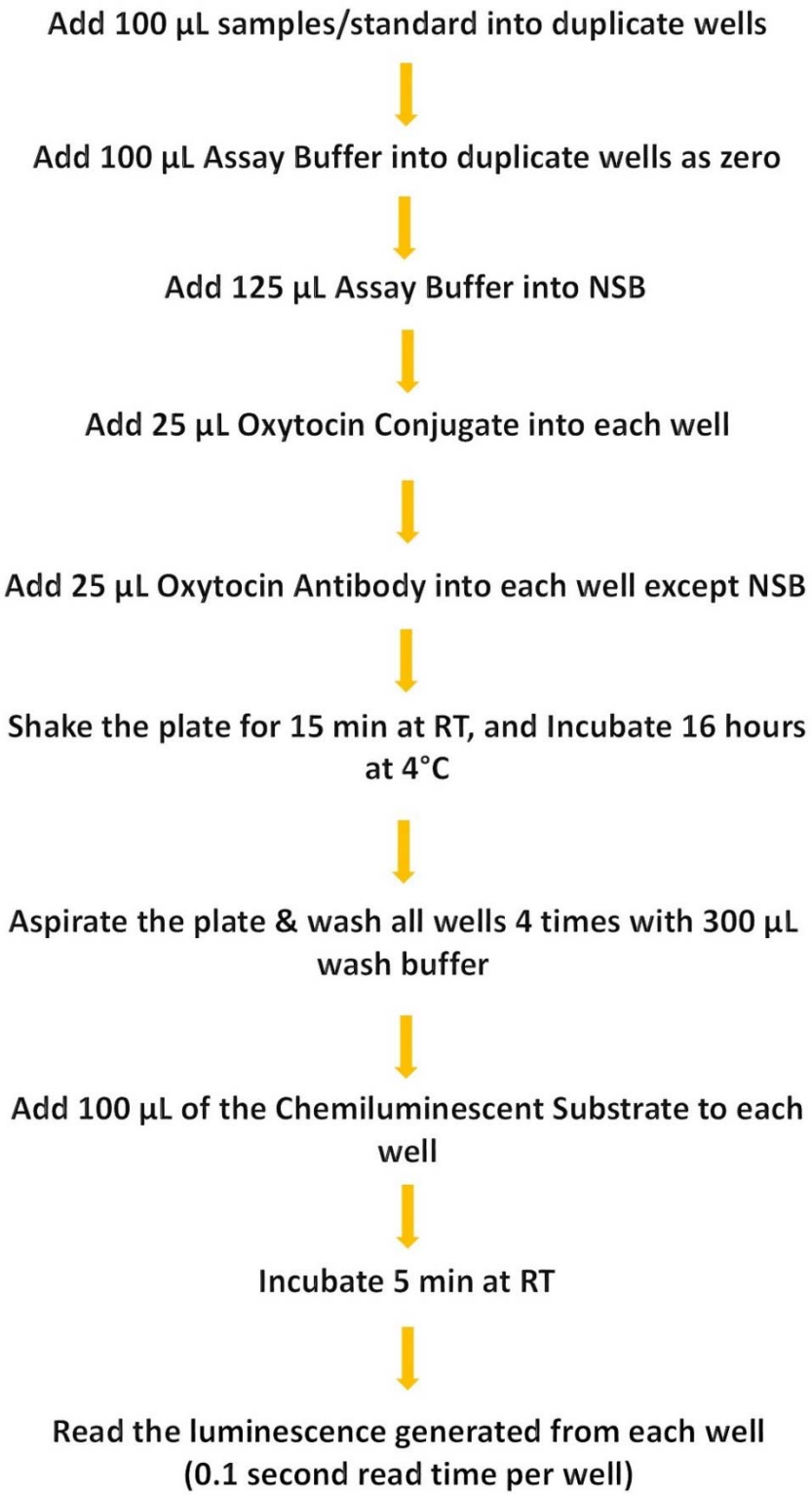
Item	*%CV*
Intra assay	6.4, 6.9
Inter assay	8.1, 7.4

Cross Reactivity

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

Steroid	*Cross Reactivity (%)*
Oxytocin	100
Isotocin	95.90
Mesotocin	88.40
Lys^8-Vasopressin	0.14
Arg^8-Vasotocin	0.13
Arg^8-Vasopressin	0.12

Protocol summary



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