Kreative Technolabs

General Oxytocin (OT) ELISA

Cat No. KTL14842

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE:
The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of Oxytocin in serum, plasma, tissues and other biological fluids. The kit is not species-specific but a general assay kit.

REAGENTS AND MATERIALS PROVIDED:

Reagents	Quantity	Reagents	Quantity
Pre-Coated Microtiter Wells	1	Assay Diluent B	1x12mL
Standard	2	Standard Diluent	1x20ml
Detection Reagent A	1x120ul	Stop Solution	1x6mL
Detection Reagent B	1x120ul		
TMB Substrate	1x9mL	Instruction manual	1
(30X) Wash Buffer	1x20mL		
Assay Diluent A	1x12mL		

Materials Required But Not Supplied:
1 Microplate reader with 450 ± 10nm filter.
2 Precision single or multi-channel pipettes and disposable tips.
3 Microcentrifuge Tubes.
4 Deionized or distilled water.
5 Absorbent paper for blotting the microtiter plate.
6 Container for Wash Solution.
7 0.01mol/L (or 1×) Phosphate Buffered Saline(PBS), pH7.0-7.2.

Storage of the Kits:
1 For unopened kit: All the reagents should be kept according to the labels on vials. The TMB Substrate, Wash Buffer (30X) and the Stop Solution should be stored at 4°C upon receipt while the others should be at -20°C.
2 For opened kits: Once the kit is opened, the remaining reagents still need to be stored according to the above storage conditions. In addition, return the unused wells to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal.

Note:
For the expiration date of the kit, please refer to the label on the kit box. All components are stable until this expiration date. It is highly recommended to use the remaining reagents within 1 month of opening.

Sample Collection and Storage:

- **Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8^{o}C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20^{o}C or -80^{o}C for later use. Avoid repeated freeze/thaw cycles.
- **Other Biological Fluids -** Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20^{o}C or -80^{o}C for later use. Avoid repeated freeze/thaw cycles.

Note:
1 Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.

2 Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
3 When performing the assay, bring samples to room temperature.
4 It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

Reagent Preparation:

1 Bring all kit components and samples to room temperature (18-25°C) before use.
2 **Standard**: Reconstitute the Standard with 1.0mL of Standard Diluent, keep for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 1000pg/mL. Please Prepare 5 tubes containing 0.6mL Standard Diluent and use the diluted standard to produce a triple dilution series. Mix each tube thoroughly before the next transfer. Prepare a dilution series with 5 points; for example: 1000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04pg/mL, 12.35pg/mL, and the last EP tube with Standard Diluent is the blank as 0pg/mL.
3. **Detection Reagent A and Detection Reagent B -** Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute to the working concentration with **Assay Diluent A and B**, respectively (1:100).
4. **Wash Solution -** Dilute 20mL of Wash Buffer (30X) with 580mL of deionized or distilled water to prepare 600 mL of Wash Buffer (1X).
5. **TMB Substrate**: Aspirate the needed dosage of the solution with sterilized tips. Do not dump the residual solution back into the vial.

Note:

1 Do not perform a serial dilution directly in the wells.
2 Prepare standard within 15 minutes before assay. Do not dissolve the reagents at 37°C directly.
3 Detection Reagent A and B are sticky solutions, therefore slowly pipette them to reduce the volume errors.
4 Carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to pipette more than 10uL at a time to ensure accuracy.
5 The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
6 If crystals have formed in the Wash Buffer (30X), warm to room temperature and mix gently until the crystals are completely dissolved.
7 Any contaminated water or container used during reagent preparation will influence the detection result.

Sample Preparalion:

1 KREATIVE TECHNOLABS is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2 Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Samples should be diluted by PBS.
3 If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4 Tissue or cell extraction samples prepared using a chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
5 Due to the possibility of mismatching between antigens from other origin and antibodies used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
6 Samples from cell culture supernatant may not be detected by the kit due to influence from factors such as cell viability, cell number and/or sampling time.
7 Fresh samples that have not been stored for extended periods of time are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and give inaccurate or incorrect results.

Assay Procedure:

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard, 1 well for blank. Add 50uL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively.

2. Add 50uL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended).

3. Cover with a Plate sealer. Incubate for 1 hour at 37°C.

4. Aspirate the solution and wash with 350uL of (1X) Wash Buffer to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by tapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

5. Add 100uL of Detection Reagent B working solution to each well.

6. Incubate for 30 minutes at 37°C after covering it with the Plate sealer.

7. Repeat the aspiration/wash process for total 5 times as conducted in step 4.

8. Add 90uL of TMB Substrate to each well. Cover with a new Plate sealer.

9. Incubate for 10-20 minutes at 37°C (Do not exceed 30 minutes). Protect from light. The liquid will turn blue with the addition of Substrate Solution.

10. Add 50uL of Stop Solution to each well. The liquid will turn yellow with the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Remove any drops of water and fingerprints on the bottom of the plate and confirm there are no bubbles on the surface of the liquid. Run the microplate reader and conduct measurement at 450nm immediately.

Note:

1 **Assay preparation**: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Remaining wells should be resealed and stored at -20°C.

2 **Samples or reagents addition**: **Please use the freshly prepared Standard.** Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. In addition, use separated reservoirs for each reagent.

3. **Incubation**: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips dry at any time during the assay. Incubation time and temperature must be controlled.

4. **Washing**: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting and remove any drops of water and fingerprints on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.

5. **Controlling of reaction time**: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. Please protect it from light.

7. The environment humidity may have an effect on the results obtained from the kit. If the humidity in your facility is less than 60%, using a humidifier is recommended.

Test Principle:

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to OT has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled OT and unlabeled OT (Standards or samples) with the pre-coated antibody specific to OT. After incubation the unbound conjugate is washed off. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of OT in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of OT in the sample.

Calculation of Result:

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between Oxytocin concentration in the sample and the assay signal intensity.

Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log or

semi-log graph paper, with the log of Oxytocin concentration on the y-axis and absorbance on the x-axis. Draw the best fit straight line through the standard points, or it can be determined by regression analysis. Using ploting software, (for instance, curve expert 1.30), is also recommended.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Detection Range:
12.35-1000pg/ml. The standard curve concentrations used for the ELISA's were 1000pg/mL, 333.33pg/mL, 111.11pg/mL, 37.04pg/mL, 12.35pg/mL.

Sensitivity:
The minimum detectable dose of OT is typically less than 5.27pg/mL.
The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity:
This assay has high sensitivity and excellent specificity for detection of Oxytocin.
No significant cross-reactivity or interference between OT and analogues was observed.

Precision:
Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Oxytocin were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Oxytocin were tested on 3 different plates, 8 replicates in each plate.
CV(%) = SD/meanX100
Intra-Assay: CV<10%
Inter-Assay: CV<12%

Recovery
Matrices listed below were spiked with certain level of Oxytocin and the recovery rates were calculated by comparing the measured value to the expected amount of Oxytocin in samples.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	90-101	96
EDTA Plasma(n=5)	79-98	90
Heparin Plasma(n=5)	90-99	94

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of Oxytocin and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation

Sample	1:2	1:4	1:8	1:16
Serum(n=5)	98-105%	86-101%	85-93%	81-98%
EDTA Plasma(n=5)	82-96%	79-94%	84-97%	85-92%
Heparin Plasma(n=5)	80-91%	88-104%	91-99%	80-95%

Stability:
The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

Note: To minimize unnecessary influences on the performance, operation procedures and lab conditions, especially room temperature, air humidity, and incubator temperatures should be strictly regulated. It is also strongly suggested that the whole assay is performed by the same experimenter from the beginning to the end.