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User's Manual

Oxytocin ELISA kit

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RUO

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.

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PRODUCT INFORMATION

Intended Use

The Oxytocin ELISA kit is a competitive immunoassay for the quantitative determination of Oxytocin in samples.

General Description

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- Vasopressin, maintain renal water and sodium balance.

Oxytocin: H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH2

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.

Principles of Testing

The kit uses a polyclonal antibody to Oxytocin to bind, in a competitive manner, the Oxytocin in the standard or sample or an alkaline phosphatase molecule which has Oxytocin covalently attached to it. After a simultaneous incubation at 4°C the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of Oxytocin in either standards or samples. The measured optical density is used to calculate the concentration of Oxytocin.

Reagents And Materials Provided

1. Goat anti-Rabbit IgG Microtiter Plate, 96 wells

A plate using break-apart strips coated with goat antibody specific to rabbit IgG.

2. Oxytocin Conjugate, 5 mL

A blue solution of alkaline phosphatase conjugated with Oxytocin.

3. Oxytocin Antibody, 5 mL

A yellow solution of a rabbit polyclonal antibody to Oxytocin.

4. Assay Buffer, 27 mL

Buffer containing proteins and sodium azide as preservative.

5. Wash Buffer Concentrate, 27 mL

Tris buffered saline containing detergents.

6. Oxytocin Standard, 0.5 mL

A solution of 10,000 pg/mL Oxytocin.

7. pNpp Substrate, 20 mL

A solution of p-nitrophenylphosphate in buffer. Ready to use.

8. Stop Solution, 5 mL

A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic.

9. Plate Sealer, 1 each
10. Oxytocin Assay Layout Sheet, 1 each

Materials Required But Not Supplied

1. Deionized or distilled water.
2. Precision pipets for volumes between 5 µL and 1,000 µL.
3. Repeater pipets for dispensing 50 and 200 µL.
4. Disposable beaker for diluting buffer concentrates.
5. Graduated cylinders.
6. Adsorbent paper for blotting.
7. Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.
8. Acetonitrile, anhydrous (>99%).
9. Trifluoroacetic acid (>99%).
10. C-18 resin cartridge/column.

Storage

All components of this kit, except the conjugate and standard, are stable at 4°C until the kit's expiration date. The conjugate and standard must be stored frozen at -20°C upon receipt.

Specimen Collection And Preparation

The ELISA is compatible with Oxytocin samples in 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. However, the end user must verify that the recommended dilutions are appropriate for their samples. Samples containing rabbit IgG may interfere with the assay.

The extraction protocol outlined below is strongly recommended for all serum and plasma samples in addition

to all other samples that cannot be sufficiently diluted to avoid matrix interference without being too dilute to measure. Additionally, extraction of samples can serve to concentrate the analyte for aid in measurement.

Extraction efficiencies for a variety of sample matrices are listed below. For each matrix listed, 200pg/mL of oxytocin was spiked into the matrix, then extracted as per the sample extraction protocol and read in the assay. The efficiency of extraction was calculated as the amount returned off the standard curve divided by the theoretical amount (200pg/mL) x 100.

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 (1mg/mL blood) tubes containing Aprotinin (500 KIU/mL of blood). Centrifuge the samples at 1,600 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.

Extraction of the sample should be carried out using a similar protocol to the one described below. For a 200 mg C18 Sep-Pak column we suggest a sample volume no greater than 3 mL.

1. Add an equal volume of 0.1% trifluoroacetic acid in water (TFA-H2O) to the sample. Centrifuge at 17,000 x g for 15 minutes at 4°C to clarify and save the supernatant.
2. Equilibrate a 200 mg C18 Sep-Pak column with 1 mL of acetonitrile, followed by 10-25 mL of 0.1% TFA-H2O.
3. Apply the supernatant to the Sep-Pak column and wash with 10-20 mL of 0.1% TFA-H2O. Discard wash.
4. Elute the sample slowly by applying 3 mL of a solution comprised of 60% acetonitrile and 40% of 0.1% TFA-H2O. Collect the eluant in a plastic tube.
5. Evaporate to dryness using a centrifugal concentrator under vacuum. Evaporation under cold temperature is recommended. Store at -20°C.
6. Reconstitute with Assay Buffer and measure immediately. You will need to have at least 250 µL volume (upon reconstitution) per sample in order to have enough material to run duplicates (n=2 per sample). Please note that upon reconstitution inso luble 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.

Reagent Preparation

1. Oxytocin Standard

Allow the 10,000 pg/mL Oxytocin standard solution to warm to room temperature. Label seven 12 x 75 mm glass tubes #1 through #7. Pipet 1 mL of standard diluent (Assay Buffer or Tissue Culture Media) into tube #1. Pipet 500 µL of standard diluent into tubes #2 through #7. Remove 100 µL of buffer from tube #1. Add 100 µL of the 10,000 pg/mL standard to tube #1. Vortex thoroughly. Add 500 µL of tube #1 to tube #2 and vortex thoroughly. Add 500 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #7.

The concentration of Oxytocin in tubes #1 through #7 will be 1,000, 500, 250, 125, 62.5, 31.2, and 15.6 pg/mL respectively. See Oxytocin Assay Layout Sheet for dilution details. Diluted standards should be used within 60 minutes of preparation.

2. Oxytocin Conjugate

Allow the conjugate to warm to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

3. Wash Buffer

Prepare the Wash Buffer by diluting 5 mL of the supplied concentrate with 95 mL of deionized water. This can be stored at room temperature until the kit expiration or for 3 months, whichever is earlier.

Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening. All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4°C.
2. Pipet 100 µL of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #7 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells
5. Pipet 50 µL of Assay Buffer into the NSB wells.
6. Pipet 50 µL of the blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of the yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

8. Tap the plate gently to mix. Seal the plate and incubate at 4°C for 18-24 hours.
9. Empty out the contents of the wells and wash by adding 400 µL of wash solution to each well. Repeat the wash 2 more times for a total of 3 Washes.
10. After the final wash, empty or aspirate the wells, and firmly tap the plate dry on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 µL of the blue Conjugate to the TA wells.
12. Add 200 µL of the pNpp Substrate solution to every well. Incubate at room temperature for 1 hour without shaking.
13. Add 50 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the 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 Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

Quality Control

Total Activity Added = 0.803 x 10 = 8.03

%NSB = 0.0%

%B0/TA = 7.56%

Quality of Fit = 1.0000*

20% Intercept = 1791pg/mL

50% Intercept = 228pg/mL

80% Intercept = 43pg/mL

(* Calculated from 4 parameter logistics curve fit)

Calculation

Several options are available for the calculation of the concentration of Oxytocin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program. Such software is often supplied by plate reader manufacturers.

Typical Standard Curve

The results shown below are for illustration only and should not be used to calculate results.

Typical standard curves are shown below. These curves must not be used to calculate Oxytocin concentrations; each user must run a standard curve for each assay.

Precision

Intra-assay precision was determined by assaying 20 replicates of three controls containing oxytocin in a single assay.

Oxytocin (pg/mL)%CV

39. 12.6

121 .4 10.2

363 .7 13.3

Inter-assay precision was determined by measuring controls of varying oxytocin concentrations in multiple assays (n=17) over several days.

Oxytocin (pg/mL)%CV

47. 0 20.9

145 .1 16.5

397 .2 11.8

Sensitivity

15.0 pg/mL

Linearity

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 spiked concentration was determined from a standard curve and the sample determined to be linear within a range of 100% ± 15% relative to a designated dilution.

Recovery

Oxytocin concentrations were measured in a variety of different samples including tissue culture media and human breast milk. Oxytocin was spiked into the undiluted samples which were diluted with the appropriate diluent and then assayed in the kit. The following results were obtained:

Precautions

1. Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up.
2. Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.
3. The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg2+ and Zn2+ ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.
4. 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.
5. The Oxytocin Standard provided is supplied in ethanolic buffer at a pH optimized to maintain Oxytocin integrity. Care should be taken in handling this material because of the known and unknown effects of Oxytocin.

References

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