

OXYTOCIN ANTISERUM
Developed in RabbitProduct No. **O3882**

This antiserum is developed using oxytocin-porcine thyroglobulin as immunogen. The product is lyophilized, diluted antiserum.*

Description

Oxytocin (OT) is a nonapeptide (approx. 1,000 Da) synthesized in the hypothalamus in its large precursor form, oxytocin-neurophysin (OT-Np), which is then processed in a series of post-translational steps and finally secreted from the neurohypophysis. Over the last decade, it has become apparent that OT is also present in the ovarian tissue of ruminants, pigs and humans. It has been ultrastructurally located in granules within the large luteal cells that originate from the granulosa compartment of the preovulatory follicle. OT content in bovine corpus luteum is highest early in the estrus cycle, whereas progesterone levels persist to later stages of the luteal phase. Oxytocin is involved in milk ejection reflex and uterine contraction, while vasopressin (VP) is mainly implicated in water metabolism. It is now evident that these neuropeptides may have additional functions in the central nervous system as well as in a variety of peripheral tissues. Neurohypophysis peptides have been demonstrated in a number of peripheral, mainly endocrine, tissues. Thus, there is a growing need for very specific immunological tools to distinguish between these very closely related neurohypophysial hormones. Oxytocin is the most potent uterotonic agent known and is used to induce labor.³ Yet, endogenous circulating OT does not appear to participate in the induction of labor. To establish whether OT peptide exists in uterine tissue, one can assay the amounts of uterine immunoreactive OT (ir-OT) by radioimmunoassay (RIA).¹ Antiserum to oxytocin is also useful for detection of oxytocin in formalin-fixed, paraffin-embedded sections of human neurohypophysis.

Reconstitution and Dilution

1. Buffer Solution: 0.02 M Barbituric acid (Sigma Product No. B0625), 0.14 M NaCl (Sigma Product No. S9625), 0.01 M sodium EDTA (Sigma Product No. E4884) containing 0.2% bovine IgG (Sigma Product No. I5506) and 0.016 mg/ml L-cysteine (Sigma Product No. C7755), pH 9.0.
2. Stock Solution: To one vial of lyophilized powder add 1.0 ml of buffer solution. Rotate vial gently until powder is dissolved.
3. Working Solution: To obtain the number of tests indicated on the vial further dilute the reconstituted antiserum 10-fold with buffer solution.

Storage

Prior to reconstitution, store at 2-8°C. After reconstitution, the stock solution should be separated into aliquots and frozen. The working dilution of the antiserum should be discarded if unused within 12 hours. Repeated freezing and thawing is **not** recommended.

Tests Per Vial

The number of tests per vial is determined by Sigma Immunochemicals utilizing the following polyethylene glycol separation ¹²⁵I radioimmunoassay (RIA) protocol, where 0.1 ml of reconstituted and diluted antiserum has been found to bind 30-50% of 1-2 picograms of iodinated oxytocin.

The number of tests per vial and subsequent lot specific data indicate the performance of the antiserum in the assay system utilized by Sigma Immunochemicals. It is recommended that the antiserum first be evaluated in the particular assay system chosen due to differences in assay systems and procedures.

Reagents

- (A) Standard: Prepare a stock standard solution of 1 mg/ml oxytocin (Sigma Product No. O6379) in buffer solution. Keep frozen aliquots at -20°C . Thaw one sample for each assay. Prior to the assay dilute an aliquot of the stock solution in buffer solution to 20 ng/ml and continue with serial dilutions to: 10, 5, 2.5, 1.25, 0.6, 0.3, 0.15, 0.075, and 0.039 ng/ml.
- (B) Antiserum to Oxytocin: Dilute with buffer according to recommended working dilution.
- (C) Radiolabelled Tracer: Freshly prepared solution of 20-40 pg/ml ^{125}I -Oxytocin with specific activity of approx. 2000 Ci/mmol (approx. 100,000 - 200,000 dpm/ml).
- (D) Polyethylene Glycol Solution: 30% (w/v) PEG, M.W. approx. 6,000, in buffer.
- (E) Polystyrene tubes.

RIA Protocol

1. Pipette 0.05 ml of standards (A) into polystyrene tubes. Prepare a zero control and a blank tube, each containing 0.05 ml of buffer.
2. Add 0.1 ml of diluted antiserum (B) to all tubes except the blank tube.
3. Incubate at room temperature for 30 minutes.
4. Add 0.05 ml of ^{125}I -oxytocin solution (C) (1-2 pg/ml) to each tube.
5. Prepare two empty tubes to the total count (T). Add 0.05 ml ^{125}I -oxytocin solution (C) to these tubes and set aside at room temperature.
6. Vortex and incubate remaining tubes at room temperature for 18-24 hours.
7. Add 0.2 ml of polyethylene glycol (D) solution to all tubes except T. Mix well.
8. Centrifuge all tubes, except T, at 1,000 g at 4°C for 15 minutes.
9. Aspirate the supernatants.
10. Count the precipitates in a gamma counter.

Specificity

Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in Sigma's polyethylene glycol separation RIA system is as follows:

Cross-Reactant	% Cross-Reactivity
Oxytocin	100
[Arg ⁸]-Vasopressin	<0.1
[Lys ⁸]-Vasopressin	<0.1
Somatostatin	<0.1
Isotocin	<10.0
LHRH	<0.1
hANP	<0.1
Rat ANP	<0.1
Tocinoic Acid	<0.1
[Arg ⁸]-Vasotocin	<0.1

Sensitivity

Sensitivity is defined at the 90% intercept of a B/B_0 standard curve. Using Sigma's RIA system, the sensitivity has been found to be <5 pg oxytocin per tube.

Affinity Constant

The affinity constant (K_a) is determined by a Scatchard plot using Sigma's RIA system.
 $K_a = 5.0 \times 10^9$ to 5.0×10^{10} L/mole.

References

1. Robert, F., et al., J. Neuroimmunol., **9**, 205 (1985).
2. Burgeon, E., et al., J. Neuroimmunol., **31**, 235 (1991).
3. Lefebvre, D., et al., Science, **256**, 1553 (1992).

* Each vial contains no more than 20 mg Polyvinylpyrrolidone (PVP). Due to the PVP content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

12/98

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.

Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.

Please see reverse side of the invoice or packing slip.