

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Description

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

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 405 nm.

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.

For further explanation of the principles and practice of immunoassays please see the excellent books by Chard¹ or Tijssen².

Introduction

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 [1-6] 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^{1,2}, may be involved in neuropsychiatric disorders³, social/sexual behavior⁴ and is important in male reproductive physiology^{5,6}. Oxytocin and the related neurohypophysial peptide, Arg⁸-Vasopressin, maintain renal water and sodium balance⁷.

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 isoleucine⁸. 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 pathway⁸.

Precautions

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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.

DRG[®] Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO**Materials Supplied**

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

Storage

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

Materials Needed 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.

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

Sample Handling

The Oxytocin 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. Please refer to the *Sample Recovery* recommendations for details of suggested dilutions.

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). Data illustrating the benefits of extraction are outlined below.

Sample	Neat, Unspiked (pg/mL)	Extracted, Unspiked (pg/mL)	Extracted, Spiked (200 pg/mL)	Extraction Efficiency
Human EDTA Plasma	2,761	3.4	169	83
Human Serum	106	30	236	103

Because of the labile nature of Oxytocin we recommend several precautions in collecting and analyzing samples.

Blood samples should be drawn into chilled EDTA (1mg/mL blood) or serum 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 (TFA) in water 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 in water.
3. Apply the supernatant to the Sep-Pak column and wash with 10-20 mL of 0.1% TFA in water. Discard wash.
4. Elute the sample slowly by applying 3 mL of acetonitrile: 0.1% TFA in water 60:40. Collect the eluate in a plastic tube.
5. Evaporate to dryness using a centrifugal concentrator under vacuum. Store at -20°C.
6. Reconstitute with Assay Buffer* and continue immediately with the Assay Procedure.

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.

DRG[®] Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

Procedure for clarification of milk

1. Centrifuge the sample at 10,000 rcf (relative centrifugal force) for 15 minutes.
2. Using a syringe, carefully pierce through the top layer and aspirate the lower layer of supernatant.
3. Centrifuge supernatant at 10,000 rcf for 15 minutes.
4. Repeat #2 and centrifuge resulting supernatant at 10,000 rcf for 15 minutes. Sample should now be ready to assay, following dilution in Assay Buffer.

Procedural Notes

1. Do not mix components from different kit lots or use reagents beyond the kit expiration date.
2. Allow all reagents to warm to room temperature for at least 30 minutes before opening.
3. Standards can be made up in either glass or plastic tubes.
4. Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.
5. Pipet standards and samples to the bottom of the wells.
6. Add the reagents to the side of the well to avoid contamination.
7. This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided.
8. **Care must be taken to minimize contamination by endogenous alkaline phosphatase.** Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands.
9. **Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.**

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

Reagent Preparation

1. Oxytocin Standard

Allow the 10,000 pg/mL Oxytocin standard solution to warm to room temperature. Label five 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.

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

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.

Calculation of Results

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 4 parameter logistic curve fitting program. If data reduction software is not readily available, the concentration of Oxytocin can be calculated as follows:

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:
Average Net OD = Average Bound OD - Average NSB OD
2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:
Percent Bound = Net OD / Net Bo OD x 100
3. Using Logit-Log paper plot Percent Bound versus Concentration of Oxytocin for the standards. Approximate a straight line through the points. The concentration of Oxytocin in the unknowns can be determined by interpolation.

Typical Results

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

Sample	Mean OD - (Blank)	Average Net OD	Percent Bound	Oxytocin (pg/mL)
Blank OD	(0.083)			
TA	0.929			
NSB	-0.004	0.000	0%	
Bo	0.484	0.488	100%	0
S1	0.056	0.060	12.3%	1,000
S2	0.098	0.102	20.9%	500
S3	0.159	0.163	33.4%	250
S4	0.235	0.239	48.9%	125
S5	0.326	0.330	67.6%	62.5
S6	0.392	0.396	81.1%	31.2

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

S7	0.443	0.447	91.6%	15.6
Unknown 1	0.142	0.146	29.9%	300
Unknown 2	0.430	0.434	88.9%	20

Calibration

Calibration to the NIBSC/WHO Oxytocin 4th International Standard 76/575 has been determined. To convert sample values obtained in the Oxytocin EIA Kit to this NIBSC/WHO Oxytocin Standard, use the equation below.

NIBSC/WHO 76/575 value (pg/mL) = Obtained Oxytocin value (pg/mL) x 0.84.

Typical Quality Control Parameters

Total Activity Added	=	0.929 x 10 = 9.29
%NSB	=	0.0%
%Bo/TA	=	5.25%
Quality of Fit	=	1.0000 (Calculated from 4 parameter logistics curve fit)
20% Intercept	=	519 pg/mL
50% Intercept	=	123 pg/mL
80% Intercept	=	34 pg/mL

Performance Characteristics

The following parameters for this kit were determined using the guidelines listed in the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols¹¹.

Sensitivity

Sensitivity was calculated by determining the average optical density bound for sixteen (16) wells run as Bo, and comparing to the average optical density for sixteen (16) wells run with Standard #7. The detection limit was determined as the concentration of Oxytocin measured at two (2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo	=	0.490 ± 0.025
Average Optical Density for Standard #7	=	0.423 ± 0.027
Delta Optical Density (0-15.62 pg/mL)	=	0.490 - 0.423 = 0.067
2 SD's of the Zero Standard	=	2 x 0.025 = 0.05
Sensitivity =	0.05 / 0.067 x 15.62 pg/mL	= 11.7 pg/mL

Linearity

A sample containing 800 pg/mL Oxytocin was serially diluted five (5) times 1:2 with kit Assay Buffer and measured in the assay. The data was plotted graphically as actual Oxytocin concentration versus measured Oxytocin concentration. The line obtained had a slope of 0.99959 with a correlation coefficient of 1.0626.

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

Precision

Intra-assay precision was determined by taking samples containing low, medium and high concentrations of Oxytocin and running these samples multiple times (n=24) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of Oxytocin in multiple assays (n=8).

The precision numbers listed below represent the percent coefficient of variation for the concentrations of Oxytocin determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	Oxytocin (pg/mL)	Intra-Assay %CV	Inter-Assay %CV
Low	21.4	9.1	
Medium	81.2	8.7	
High	300.6	12.4	
Low	19.3		14.5
Medium	84.7		8.7
High	300.3		5.2

Cross Reactivities

The cross reactivities for a number of related compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer at concentrations from 10,000 to 1 pg/mL. These samples were then measured in the Oxytocin assay, and the measured Oxytocin concentration at 50% B/Bo calculated. The % cross reactivity was calculated by comparison with the actual concentration of cross reactant in the sample and expressed as a percentage.

Compound	Cross Reactivity	Compound	Cross Reactivity
Mesotocin	131%	Somatostatin	< 0.02%
Oxytocin	100%	Met-Enkephalin	< 0.02%
Arg ⁸ -Vasotocin	42.2%	VIP	< 0.02%
Oxytocin-SH	15.4%	Lys ⁸ -Vasopressin	< 0.02%
Arg ⁸ -Vasopressin-SH	0.6%	Arg ⁸ -Vasopressin	< 0.02%
Lys ⁸ -Vasopressin-SH	0.6%	α-ANP	< 0.02%
Ser ⁴ , Ile ⁸ -Oxytocin	< 0.02%	Growth Hormone	< 0.02%
TRH	< 0.02%	Tocinoic acid	< 0.02%
		Melanostatin	< 0.02%

Sample Recoveries

Please refer to page 3/4 for *Sample Handling* recommendations.

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:

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

<u>Sample</u>	<u>%Recovery*</u>	<u>Recommended Dilution*</u>
Tissue Culture Media	102	Neat
Human Breast Milk	95	1:2

* See Sample Handling instructions for details.

References

1. T. Chard, "An Introduction to Radioimmunoassay & Related Techniques 4th Ed.", (1990) Amsterdam: Elsevier.
2. P. Tijssen, "Practice & Theory of Enzyme Immunoassays", (1985) Amsterdam: Elsevier.
3. G.L. Kovacs, & D.H.K. Versteeg, Ann. NY Acad. Sci., (1993), 689: 309.
4. T.R. Insel, et al., Rev. Reprod., (1997), 2(1): 28.
5. A. Frasch, et al., "Oxytocin: Cellular and Molecular Approaches", (1995) NY: Plenum Press.
6. M.M. McCarthy, & M. Altemus, Mol. Med. Today, (1997), 3(6): 269.
7. R. Ivell, et al., Adv. Exp. Med. Biol., (1997), 424: 253.
8. A. Argiolas, & M.R. Melis, "Oxytocin: Cellular and Molecular Approaches", (1995) NY: Plenum Press.
9. K.P. Conrad, et al., Ann. NY Acad. Sci., (1993), 689: 346.
10. R. Archer, & J. Chauvet, Front. Neu-roendo., (1995), 16: 237.
11. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

LIMITED WARRANTY

DRG warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.





















DRG must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if DRG is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

DRG® Oxytocin (EIA-3117)

Revised 29 July 2008 (Vers. 4.0)

RUO

Symbols used with DRG ELISAs

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità
Symbol	Portugues	Dansk	Svenska	Ελληνικά	
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη	
	Conformidade com as normas europeias	Europeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση	
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό	
					
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου	
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος	
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις	
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης	
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης	
	Fabricante	Producent	Tillverkare	Κατασκευαστής	
Distributed by					
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο	
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..	