

**Instructions for use**  
**Estradiol rat ELISA**

**REF****AR E-8800****RUO**For Research use only -  
Not for use in diagnostic  
procedures

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## **ESTRADIOL RAT ELISA**

### **INTRODUCTION**

#### **INTENDED USE**

The **Estradiol rat ELISA** is a competitive immunoassay for the quantitative measurement of estradiol in rat serum. **For research use only.**

#### **SUMMARY AND EXPLANATION**

Estradiol (E2 or 17 $\beta$ -estradiol) is an estrogenic hormone produced by the ovaries and in smaller amounts by the adrenal cortex and testes. It is the most potent female sexual hormone and is essential for maintaining normal female functions. During the oestrous cycle which can be divided into four phases (proestrus, estrus, metestrus, diestrus) estradiol concentrations rise gradually from metestrus to proestrus and fell to barely detectable levels in estrus. The maximum concentration is reached around mid-day of proestrus (1,2).

Apart from its effects on sexual characteristics it has important influence on the growth and development of the brain (3,4).

In female rodents, the determination of estradiol is a useful marker in evaluating and monitoring the state of the reproductive functions and pregnancy as well.

#### **PRINCIPLE**

The Estradiol rat ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding.

The microtiter wells are coated with an anti-Estradiol antibody. An unknown amount of estradiol present in the sample competes with an Estradiol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of Estradiol in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of free Estradiol in the sample.

#### **WARNINGS AND PRECAUTIONS**

- This kit is for research use only. For professional use only.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2 – 8°C in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Avoid contact with Stop Solution. It may cause skin irritation and burns.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

- For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

## REAGENTS

### REAGENTS PROVIDED

**AR E-8831** 96 **Microtiterplate**, 12 x 8 (break apart) strips with 96 wells; ready to use; Wells coated with anti-Estradiol antibody.

**Calibrators** - lyophilized, reconstitution required  
For reconstitution see "Reagent Preparation".

Cat. no.	Symbol	Calibrator	Concentration
<b>AR E-8801</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD A</span>	<b>Calibrator 0</b>	0 pg/ml
<b>AR E-8802</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD B</span>	<b>Calibrator 1</b>	5 pg/ml
<b>AR E-8803</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD C</span>	<b>Calibrator 2</b>	20 pg/ml
<b>AR E-8804</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD D</span>	<b>Calibrator 3</b>	80 pg/ml
<b>AR E-8805</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD E</span>	<b>Calibrator 4</b>	320 pg/ml
<b>AR E-8806</b>	<span style="border: 1px solid black; padding: 0 2px;">STANDARD F</span>	<b>Calibrator 5</b>	1280 pg/ml

**AR E-8813** INC-BUFF **Incubation Buffer**, 1 vial 7 ml, ready to use;

**AR E-8841** DILUENT **Enzyme Conjugate Diluent**, 1 vial, 30 ml, ready to use

**AR E-8840** CONJUGATE **Enzyme Conjugate**, 1 vial, 0,3 ml (100X concentrated);  
17 $\beta$ -Estradiol labeled horseradish peroxidase in buffered matrix  
see "Reagent Preparation".

**AR E-0055** SUBSTRATE **Substrate Solution**, 1 vial, 22 ml, ready to use;  
contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.

**AR E-0080** STOP-SOLN **Stop Solution**, 1 vial, 7 ml, ready to use;  
contains 2 N Hydrochloric Acid solution.

**AR E-0030** WASH-CONC 10x **Wash Solution**, 1 vial, 50 ml (10X concentrated);  
see "Reagent Preparation".

**Note:** Additional Calibrator 0 for sample dilution is available upon request.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Microcentrifuge
- A microtiter plate reader capable for endpoint measurement at 450 nm
- Calibrated variable precision micropipettes (50  $\mu$ l, 75 $\mu$ l, 200  $\mu$ l, 300  $\mu$ l).
- Microplate mixer operating more than 600 rpm
- Vortex mixer
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

### STORAGE CONDITIONS

When stored at 2°C to 8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Take care that the foil bag is sealed tightly.

Store Calibrators refrigerated, they will be stable at 2-8°C for 7 days after reconstitution. For longer storage freeze at -20°C.

## REAGENT PREPARATION

Allow the reagents and the required number of wells to reach room temperature (21-26°C) before starting the test.

### Wash Solution:

Dilute 50 mL of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml. *The diluted Wash Solution is stable for at least 3 months at room temperature (21-26°C).*

### Calibrators:

Reconstitute lyophilized calibrators with **0.5 ml deionized water** 30 min. before use.

### Enzyme Conjugate:

Immediately before use dilute Enzyme Conjugate Concentrate 1:100 in Enzyme Conjugate Diluent, for example 0.1 ml concentrated Enzyme Conjugate Concentrate + 9.9 ml Enzyme Conjugate Diluent. Mix thoroughly.

## DISPOSAL OF THE KITS

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheet.

## DAMAGED TEST KITS

In case of any severe damage of the test kit or components, the manufacturer have to be informed written, latest one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

## SPECIMEN

For determination of rat estradiol **serum samples** can be used. The procedure calls for 75 µl sample per well. The samples should assay immediately or aliquot and stored at -20°C. Avoid repeated freeze-thaw cycles. Samples expected to contain estradiol concentrations higher than the highest calibrator (1280 pg/ml) should be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results.

## ASSAY PROCEDURE

### GENERAL REMARKS

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instructions for use.

## ASSAY PROCEDURE

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Dispense **75 µl** of each **Calibrator** and **Sample** with new disposable tips into appropriate wells.
3. Dispense **50 µl** of **Incubation Buffer** into each well.
4. Incubate for **120 minutes** at room temperature on a plate shaker (> 600 rpm).
5. Immediately before use dilute the Enzyme Conjugate Concentrate 1:100 in Enzyme Conjugate Concentrate.  
Add **50 µL diluted Enzyme Conjugate** into each well.
6. Incubate for **60 minutes** at room temperature on a plate shaker (> 600 rpm)  
**Important Note:**  
Optimal reaction in this assay is markedly dependent on shaking of the microplate!
7. Discard the content of the wells and rinse the wells **4 times** with diluted **Wash Solution** (300 µl per well). Remove as much Wash Solution as possible by beating the microplate on absorbent paper.
8. Add **200 µl** of **Substrate Solution** to each well.
9. Incubate without shaking for **30 minutes** in the dark.
10. Stop the reaction by adding **50 µl** of **Stop Solution** to each well.
11. Determine the absorbance of each well at 450 nm. It is recommended to read the wells within 15 minutes.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of calibrators, controls and patient samples.
2. Using semi logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration from the calibration curve.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

### Example of Typical Calibrator Curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard	Optical Units (450nm)
Calibrator 0 (0 pg/mL)	2.945
Calibrator 1 (5 pg/mL)	2.667
Calibrator 2 (20 pg/mL)	2.114
Calibrator 3 (80 pg/mL)	0.989
Calibrator 4 (320 pg/mL)	0.311
Calibrator 5 (1280 pg/mL)	0.129

## EXPECTED VALUES

Each laboratory should establish its own normal ranges.

## **PERFORMANCE CHARACTERISTICS**

### **ANALYTICAL SENSITIVITY**

The lowest analytical detectable level of Estradiol that can be distinguished from the Zero Calibrator is 2.5 pg/ml at the 2SD confidence limit.

### **SPECIFICITY (CROSS REACTIVITY)**

The following materials have been evaluated for cross reactivity. The percentage indicates cross reactivity at 50% displacement compared to progesterone.

<b>Steroid</b>	<b>% Cross reaction</b>
Androstenedione	< 0.1
17-Hydroxyprogesterone	< 0.1
Corticosterone	< 0.1
Estriol	0.4
Estrone	4.2
Pregnenolone	< 0.1
E2-3-Glucuronide	3.8
E2-3-Sulphate	3.6
E2-17-Glucuronide	< 0.1
Progesterone	< 0.1
Testosterone	< 0.1

### **ASSAY DYNAMIC RANGE**

The range of the assay is between 2.5 – 1280 pg/ml.

### **REPRODUCIBILITY**

#### **Intra-Assay**

The intra-assay variation was determined by 20 replicate measurements of three serum samples within one run. The within-assay variability is shown below:

	<b>Serum 1</b>	<b>Serum 2</b>	<b>Serum 3</b>
<b>Mean (pg/mL)</b>	29.9	118.9	246.3
<b>SD</b>	1.8	3.6	7.8
<b>CV (%)</b>	6.1	3.0	3.2
<b>n =</b>	20	20	20

#### **Inter-Assay**

The inter-assay (between-run) variation was determined by duplicate measurements of three serum samples in 11 different tests.

	<b>Serum 1</b>	<b>Serum 2</b>	<b>Serum 3</b>
<b>Mean (pg/mL)</b>	23.6	104.7	226.8
<b>SD</b>	1.6	4.1	16.0
<b>CV (%)</b>	7.0	4.0	7.1
<b>n =</b>	11	11	11

## RECOVERY

Using the Calibrator Matrix two spiking solutions of 100 ng/mL and 10 ng/mL were prepared. 1000 µL of one serum was spiked with 2, 3.5 and 5 µL of the 100 ng/mL solution, and 1000 µL of two sera spiked with 5, 10 and 15 µL of the 10 ng/mL solution, leaving the serum matrices intact. All samples were measured by the Estradiol rat ELISA procedure.

Sample	Spiking (pg/mL)	Measured (pg/mL)	Expected (pg/mL)	Recovery ( % )
1	-	20.7	-	-
	200	211.1	220.7	96%
	350	298.7	370.7	81%
	500	444.8	520.7	85%
2	-	19.4	-	-
	50	68.6	69.4	99%
	100	111.9	119.4	94%
	150	143.4	169.4	85%
3	-	11.2	-	-
	50	71.6	61.2	117%
	100	128.4	111.2	115%
	150	156.3	161.2	97%

## LINEARITY

Three serum samples were assayed undiluted and diluted with the zero calibrator.

Serum	Dilution	Measured (pg/mL)	Expected (pg/mL)	Linearity (%)
1	-	196,7	./.	./.
	1 in 2	80,3	98,4	82%
	1 in 4	44,1	49,2	90%
	1 in 8	25,7	24,6	104%
2	-	231,3	./.	./.
	1 in 2	124,2	115,7	107%
	1 in 4	67,6	57,8	117%
	1 in 8	33,6	28,9	116%
3	-	359,9	./.	./.
	1 in 2	182,1	180,0	101%
	1 in 4	95,4	90,0	106%
	1 in 8	49,4	50,0	99%

## LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

## DRUG INTERFERENCES

Until now no substances (drugs) are known influencing the measurement of rat estradiol in serum and plasma. Lipemic and haemolysed samples can cause false results.

## **LEGAL ASPECTS**

### **Reliability of Results**

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

### **Liability**

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.













Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

## **REFERENCES**

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3. Zamani, M.R, Desmond, N.L, Levy, W.B..  
Estradiol modulates long term synaptic depression in female rat hippocampus.  
J. Neurophysiol (2000); 84: 1800 - 1808.
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### **Symbols:**

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		For research use only!