

## 17 $\beta$ -Estradiol high sensitivity ELISA kit

Catalog # ADI-900-174

96 Well Enzyme-Linked Immunosorbent Assay Kit  
For use with serum and plasma



Reagents require separate storage conditions.



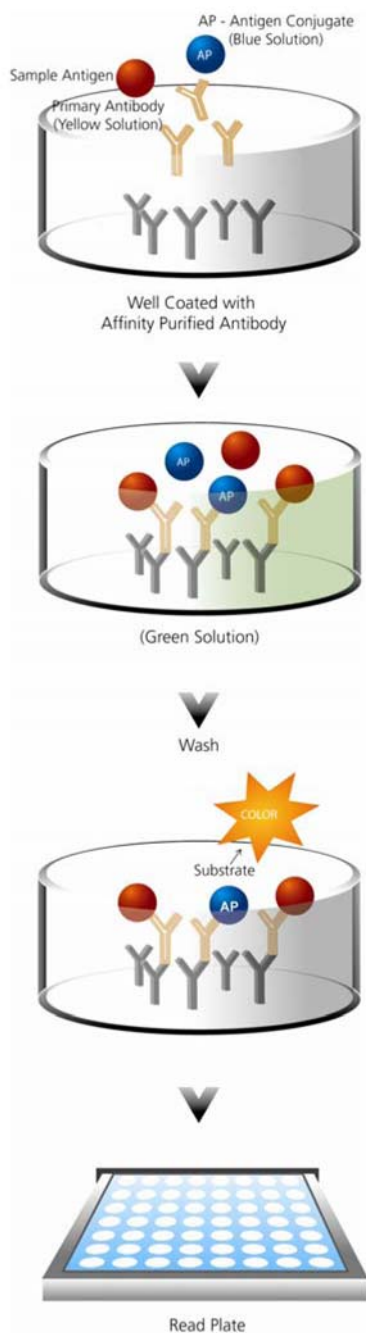
Check our website for additional protocols, technical notes and FAQs.



For proper performance, use the insert provided with each individual kit received.

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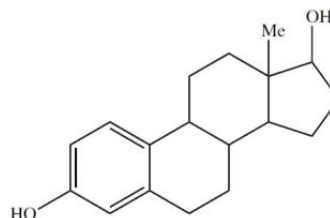
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## Introduction

17 $\beta$ -Estradiol (E2) is a female sex steroid hormone produced mainly in the ovaries, by the placenta during pregnancy, and to a lesser extent in the adrenal cortices, testes, and peripheral tissues<sup>1-3</sup>. The hormone is synthesized enzymatically from acetate, cholesterol, progesterone and testosterone<sup>1</sup>. In addition to estradiol's well described anatomic and physiological regulation of reproduction<sup>2,3</sup> and secondary sex characteristics, it also influences diverse activities such as bone growth, brain development and maturation, and the intracellular concentration of calcium<sup>4</sup> and certain second messenger molecules<sup>5</sup>. For an extensive review of the non-reproductive actions of estradiol see the Ciba Foundation Symposium, 1995.

Estradiol-17 $\beta$



## Principle

- Standards and samples are added to wells coated with a DxS IgG antibody. A blue solution of estradiol-17 $\beta$  conjugated to alkaline phosphatase is then added, followed by a yellow solution of sheep polyclonal antibody to estradiol-17 $\beta$ .
- During a simultaneous incubation at room temperature the antibody binds in a competitive manner, the estradiol-17 $\beta$  in the sample or conjugate. The plate is washed, leaving only bound estradiol-17 $\beta$ .
- pNpp substrate solution is added. The substrate generates a yellow color when catalyzed by the alkaline phosphatase on the estradiol-17 $\beta$  conjugate.
- Stop solution is added. The yellow color is read at 405 nm. The amount of signal is indirectly proportional to the amount of estradiol-17 $\beta$  in the sample.



Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.



The standard should be handled with care due to the known and unknown effects of the antigen.



Avoid contamination by endogenous alkaline phosphatase. Do not expose reagents or supplies to bare skin.



Activity of conjugate is affected by concentrations of chelators > 10mM (such as EDTA and EGTA).



Stop solution is caustic. Keep tightly capped.



Reagents require separate storage conditions.

## Materials Supplied

1. **Assay Buffer Low BSA**  
27 mL, Product No. 80-2079  
Tris buffer containing proteins and sodium azide
2. **17 $\beta$ -Estradiol Standard**  
0.5 mL, Product No. 80-0114  
A solution of 300,000 pg/mL Estradiol-17 $\beta$
3. **Donkey anti-Sheep IgG Microtiter Plate**  
One plate of 96 wells, Product No. 80-0045  
A clear plate of break-apart strips coated with a donkey anti-sheep polyclonal antibody
4. **Estradiol-17 $\beta$  Antibody**  
5 mL, Product No. 80-2077  
A yellow solution of sheep polyclonal antibody to Estradiol-17 $\beta$
5. **Estradiol-17 $\beta$  Conjugate**  
5 mL, Product No. 80-2078  
A blue solution of Estradiol-17 $\beta$  conjugated to alkaline phosphatase
6. **Wash Buffer Concentrate**  
27 mL, Product No. 80-1286  
Tris buffered saline containing detergents
7. **pNpp Substrate**  
20 mL, Product No. 80-0075  
A solution of p-nitrophenyl phosphate
8. **Stop Solution**  
5 mL, Product No. 80-0247  
A solution of trisodium phosphate in water
9. **Estradiol-17 $\beta$  Complete Assay Layout Sheet**  
1 each, Product No. 30-0281
10. **Plate Sealer**  
1 each, Product No. 30-0012

## Storage

The **Estradiol-17 $\beta$  Antibody and Conjugate** are stable at -20°C. All other components of this kit are stable at 4°C until the kit's expiration date. The Antibody and Conjugate **should** be stored at -20°C upon receipt.

## Other Materials Needed

1. Deionized or distilled water
2. Precision pipets for volumes between 5  $\mu\text{L}$  and 1,000  $\mu\text{L}$
3. Repeater pipet for dispensing 50  $\mu\text{L}$  and 200  $\mu\text{L}$
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Microplate shaker
7. Lint-free paper toweling for blotting
8. Microplate reader capable of reading at 405 nm
9. 200 mg  $\text{C}_{18}$  solid phase system columns (Burdick & Jackson recommended)
10. Vacuum manifold
11. Speedvac
12. 100% Methanol
13. Diethyl Ether
14. Data reduction software capable of analyzing data, preferably with a 4 parameter logistic curve fit.

## Sample Handling



If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.



Samples must be stored at or below  $-20^{\circ}\text{C}$  to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

For an accurate determination of total estradiol-17 $\beta$  concentration in serum and plasma samples, extract samples prior to assaying. A minimum 1:2 and 1:4 dilution is required for serum and plasma, respectively, after extraction. These are the minimum dilutions required to remove matrix interference in the assay. Pregnancy samples should be diluted at least 1:32 after extraction. This was the minimum dilution required due for samples to read within the linear range of the assay.

Samples with low levels of estradiol-17 $\beta$  may be concentrated during the extraction procedure. For example, extracting 1 mL of sample and reconstituting with 0.25 mL of the assay buffer would concentrate the sample 4 times.

Either liquid-liquid extraction or solid phase extraction methods may be used. Solid phase extraction allows for higher throughput sample processing with the use of a vacuum manifold and speedvac.

## Solid Phase Extraction

### Materials Needed:

1. Estradiol-17 $\beta$  standard to allow extraction efficiency to be accurately determined. An extraction efficiency protocol is available on our website.
2. 200 mg C<sub>18</sub> solid phase system columns (Burdick & Jackson recommended)
3. Vacuum manifold
4. Speedvac
5. 100% Methanol
6. Diethyl Ether
7. dH<sub>2</sub>O

### Procedure:

1. Condition 200 mg C<sub>18</sub> solid phase system columns on a vacuum manifold by passing 5-10 mL of 100% methanol through the columns, followed by 5-10 mL of dH<sub>2</sub>O.
2. Apply serum and plasma samples.
3. Wash columns with 5-10 mL dH<sub>2</sub>O. Allow water to drain completely from columns until dry.
4. Elute samples with 2 mL of diethyl ether.
5. Dry samples down in a speedvac for 2-3 hrs.
6. Rehydrate samples at room temperature in the assay buffer. A minimum of 250  $\mu$ L of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement.

## Liquid-Liquid Extraction

### Materials Needed:

1. Estradiol-17 $\beta$  standard to allow extraction efficiency to be accurately determined. An extraction efficiency protocol is available on our website.
2. Diethyl Ether
3. Speedvac
4. dH<sub>2</sub>O

### Procedure:

1. Add diethyl ether to serum or plasma samples at a 5:1 (v/v) ether:sample ratio.
2. Mix solutions by vortexing for 2 minutes.
3. Allow phases to separate for 2 minutes.
4. Transfer organic phase to a glass test tube containing 1 mL dH<sub>2</sub>O.
5. Vortex the mixture for 2 minutes, and allow phases to separate for 2 minutes.



Tip: Place samples in the freezer for a few minutes to help the layers separate.

- Transfer organic phase to a clean glass test tube, and dry samples down using a speed vac for 2-3 hours.
- Rehydrate samples at room temperature in the assay buffer. A minimum of 250  $\mu\text{L}$  of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement

### Sample Recoveries

The Estradiol-17 $\beta$  standard was diluted to 100 pg/mL in the assay buffer. This sample was extracted per the above solid phase extraction procedure using a 200 mg column, and then run in the assay. The mean result was 97.9 pg/mL for 98% extraction efficiency.

### Reagent Preparation



Bring all reagents to room temperature for at least 30 minutes prior to opening.



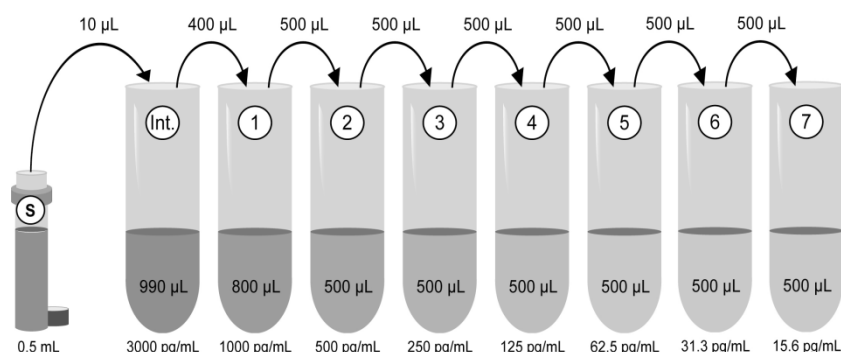
Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

#### 1. Wash Buffer

Prepare the wash buffer by diluting 5 mL of the supplied Wash Buffer 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.

#### 2. Estradiol-17 $\beta$ Standard

Allow the 300,000 pg/mL 17 $\beta$ -Estradiol standard to come to room temperature and vortex prior to use. Label seven 12 x 75 mm tubes #1 through #7 and one tube "int". Pipet 990  $\mu\text{L}$  assay buffer into tube "int" and 800  $\mu\text{L}$  into tube #1. Pipet 500  $\mu\text{L}$  assay buffer into tubes #2 through #7. Remove 10  $\mu\text{L}$  from the stock vial and add to tube "int" and vortex thoroughly. Remove 400  $\mu\text{L}$  from tube "int" and add to tube #1 and vortex thoroughly. Remove 500  $\mu\text{L}$  from tube #1 and add to tube #2. Vortex thoroughly. Continue this from tubes #3 through #7.



**Diluted standards should be used within 60 minutes of preparation.** The concentrations of Estradiol-17 $\beta$  in the tubes are labeled above.



### 3. **Conjugate 1:2 Dilution for Total Activity Measurement**

Prepare the Conjugate 1:2 Dilution by diluting 50  $\mu\text{L}$  of the supplied Conjugate with 50  $\mu\text{L}$  of Assay Buffer. The dilution should be used within three hours of preparation. **This 1:2 dilution is intended for use in the Total Activity wells ONLY.**

#### **Assay Procedure**

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 150  $\mu\text{L}$  of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100  $\mu\text{L}$  of the assay buffer into the Bo (0 pg/mL standard) wells.
3. Pipet 100  $\mu\text{L}$  of Standards #1 through #7 to the bottom of the appropriate wells.
4. Pipet 100  $\mu\text{L}$  of the samples to the bottom of the appropriate wells.
5. Pipet 50  $\mu\text{L}$  of the blue conjugate into each well except the TA and Blank wells.
6. Pipet 50  $\mu\text{L}$  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.
7. Seal the plate. Incubate at room temperature with shaking (~500 rpm\*) for two hours.
8. Empty the contents of the wells and wash by adding 400  $\mu\text{L}$  of wash buffer to every well. Repeat 2 more times for a total of **3 washes**. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
9. Pipet 5  $\mu\text{L}$  of the blue conjugate (diluted 1:2) to the TA wells.
10. Add 200  $\mu\text{L}$  of the substrate solution into each well.
11. Incubate for 1 hour at room temperature without shaking.
12. Pipet 50  $\mu\text{L}$  stop solution into each well.



Bring all reagents to room temperature for at least 30 minutes prior to opening.



Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.



All standards and samples should be run in duplicate.



Pipet the reagents to the sides of the wells to avoid possible contamination.



Prior to the addition of substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

13. After blanking the plate reader against the substrate blank, read optical density at 405 nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

\* The optimal speed for each shaker will vary and may range from 120-700 rpm. The speed must be set to ensure adequate mixing of the wells, but not so vigorously that the contents of the wells splash out and contaminate other wells.

### Calculation of Results



Make sure to adjust sample concentrations by the

Several options are available for the calculation of the concentration of Estradiol-17 $\beta$  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 Estradiol-17 $\beta$  can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

$$\text{Average Net OD} = \text{Average OD} - \text{Average NSB OD}$$

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

$$\text{Percent Bound} = \frac{\text{Net OD}}{\text{Net Bo OD}} \times 100$$

3. Plot the Percent Bound (B/Bo) versus concentration of Estradiol-17 $\beta$  for the standards. Approximate a straight line through the points. The concentration of Estradiol-17 $\beta$  of the unknowns can be determined by interpolation.

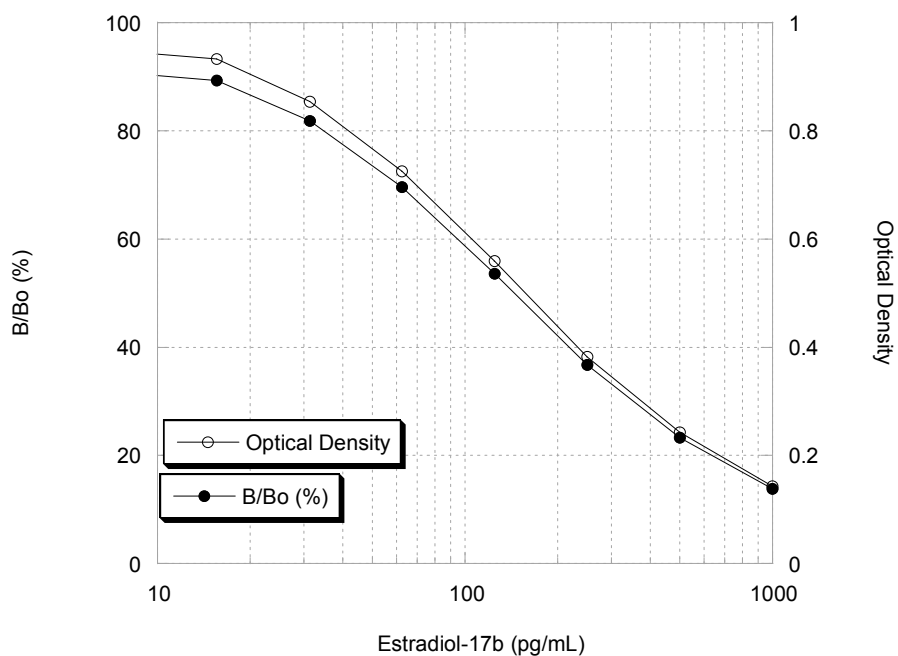
Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.



## Typical Results

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

Sample	Average Net OD	Percent Bound	Estradiol-17 $\beta$ (pg/mL)
TA	0.733	---	---
NSB	0.000	0%	---
Bo	1.044	100%	0
S1	0.143	13.8%	1000
S2	0.243	23.3%	500
S3	0.382	36.7%	250
S4	0.559	53.6%	125
S5	0.725	69.6%	62.5
S6	0.854	81.8%	31.3
S7	0.933	89.3%	15.6
Unknown 1	0.286	27.4%	391
Unknown 2	0.541	51.8%	134



## **Performance Characteristics**

### **Specificity**

The cross reactivities for a number of related compounds were determined by diluting the cross reactants in the kit assay buffer at a concentration of 100, 10, 1, and 0.1 times the high standard. These samples were then measured in the assay.

<b>Compound</b>	<b>Cross Reactivity</b>
Estrone	17.8%
Estriol	0.9%
DHT	0.12%
Ethinylestradiol-7 $\alpha$	0.043%
Dehydroisoandrosterone	0.038%
Estradiol-17 $\alpha$	0.032%
Testosterone	0.01%
Progesterone	<0.001%
Danazol	<0.001%

### **Sensitivity**

The sensitivity or limit of detection of the assay is 14.0 pg/mL. The sensitivity was determined by interpolation at 2 standard deviations below the mean signal at maximal binding (0 ng/mL) using data from 6 standard curves.

### **Linearity**

Human serum and plasma was serially diluted 1:2 in the kit assay buffer and measured in the assay. The results are shown in the table below.

<b>Dilution</b>	<b>Average % of Expected</b>		
	<b>Serum</b>	<b>Plasma</b>	<b>Pregnancy Serum</b>
Neat	92	82	-
1:2	95	86	-
1:4	100	100	-
1:8	-	-	-
1:16	-	-	116
1:32	-	-	111
1:64	-	-	100

## Precision

Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing Estradiol-17 $\beta$  in a single assay.

pg/mL	%CV
391.0	2.1
127.2	7.1
70.6	5.7

Inter-assay precision was determined by measuring buffer controls (n=13) of varying Estradiol-17 $\beta$  concentrations in multiple assays over several days.

pg/mL	%CV
380.8	8.3
123.3	9.3
65.1	14.2

## References

1. N.W. Tietz, "Textbook of Clinical Chemistry", (1986) Philadelphia, PA: W.B. Saunders Co.
2. J.K. Grant & G.H. Beastall, "Clinical Biochemistry of Steroid Hormones: Methods and Applications", (1983), Elsevier, Amsterdam.
3. M.L. Taymor, Fertility and Sterility, (1996), 65 (2): 235.
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5. E.E. Baulieu & P. Robel, "Ciba Foundation Symposium 191" (1995), John Wiley & Sons, Chichester, UK.



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### TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

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#### Global Headquarters

##### Enzo Life Sciences Inc.

10 Executive Blvd  
Farmingdale, NY 11735  
(p) 1-800-942-0430  
(f) 1-631-694-7501  
(e) [info-usa@enzolifesciences.com](mailto:info-usa@enzolifesciences.com)

##### Enzo Life Sciences (ELS) AG

Industriestrasse 17, Postfach  
CH-4415 Lause / Switzerland  
(p) +41/0 61 926 89 89  
(f) +41/0 61 926 89 79  
(e) [info-ch@enzolifesciences.com](mailto:info-ch@enzolifesciences.com)

Please visit our website at [www.enzolifesciences.com](http://www.enzolifesciences.com) for additional contact information.