Product Manual

17β-Estradiol high sensitivity ELISA Kit

Catalog number: ADI-900-174

96 Well Enzyme-Linked Immunosorbent Assay kit

For use with serum and plasma

For the latest product information, including support documentation, visit us online:
www.enzolifesciences.com

Technical Support (US): 800-942-0430
Technical Support (EU): +41 61 926 8989

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research use only. Not for use in diagnostic procedures. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON- INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

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

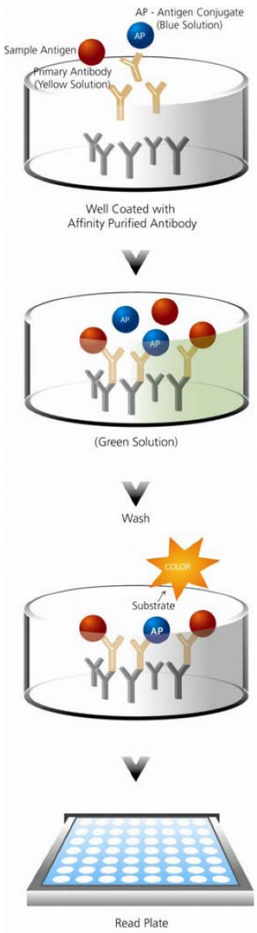
Product Manual

TABLE OF CONTENTS

Carefully note the handling and storage conditions of each kit component.

Please read entire booklet before proceeding with the assay.

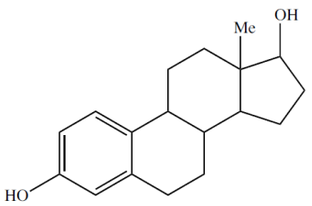
Please contact Enzo Life Sciences Technical Support if necessary.



INTRODUCTION

17β-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[1-3]. The hormone is synthesized enzymatically from acetate, cholesterol, progesterone and testosterone[1]. In addition to estradiol's well described anatomic and physiological regulation of reproduction[2,3] and secondary sex characteristics, it also influences diverse activities such as bone growth, brain development and maturation, and the intracellular concentration of calcium[4] and certain second messenger molecules[5]. For an extensive review of the non-reproductive actions of estradiol, see the Ciba Foundation Symposium,1995.

17β-Estradiol



PRINCIPLE OF THE ASSAY

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

MATERIALS SUPPLIED

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.

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

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

Stop solution is caustic. Keep tightly capped.

1. **Assay Buffer Low BSA**

   27 mL, Component number 80-2079

   Tris buffer containing proteins and sodium azide

2. **17β-Estradiol Standard**

   0.5 mL, Component number 80-0114

   A solution of 300,000 pg/mL 17β-Estradiol

3. **Donkey anti-Sheep IgG Clear Microtiter Plate**

   One plate of 96 wells, Component number 80-0045

   A clear plate of break-apart strips coated with a donkey anti-sheep polyclonal antibody

4. **17β-Estradiol Antibody**

   5 mL, Component number 80-2077

   A yellow solution of sheep polyclonal antibody to 17β-Estradiol

5. **17β-Estradiol Conjugate**

   5 mL, Component number 80-2078

   A blue solution of 17β-Estradiol conjugated to alkaline phosphatase

6. **Wash Buffer Concentrate**

   27 mL, Component number 80-1286

   Tris buffered saline containing detergents

7. **pNpp Substrate**

   20 mL, Component number 80-0075

   A solution of p-nitrophenyl phosphate

8. **Stop Solution**

   5 mL, Component number 80-0247

   A solution of trisodium phosphate in water

9. **17β-Estradiol Complete Assay Layout Sheet**

   1 each, Component number 30-0281

10. **Plate Sealer**

    1 each, Component number 30-0012

Reagents require separate storage conditions.

STORAGE

The **17β-Estradiol 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.

ADDITIONAL MATERIALS NEEDED

- Deionized or distilled water
- Precision pipets for volumes between 5 µL and 1,000 µL
- Repeater pipet for dispensing 50 µL and 200 µL
- Disposable beakers for diluting buffer concentrates
- Graduated cylinders
- Microplate shaker
- Lint-free paper toweling for blotting
- Microplate reader capable of reading at 405 nm
- 200 mg C_{18} solid phase system columns (Burdick & Jackson recommended)
- Vacuum manifold
- Speedvac
- 100% Methanol
- Diethyl Ether
- 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.

For an accurate determination of total 17β-estradiol 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 17β-estradiol may be concentrated during the extraction procedure. For example, estracting 1 mL of sample and reconstituting with 0.25 mL of the assay buffer would concentrate the sample 4 times.

Samples must be stored at or below -20˚C to avoid loss of bioactive analyte. Repeated freeze/thaw cycles should be avoided.

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. 17β-Estradiol standard to allow extraction efficiency to be accurately determined. An extraction efficiency protocol is available on our website.
2. 200 mg C_{18} solid phase system columns (Burdick & Jackson recommended)
3. Vacuum manifold
4. Speedvac
5. 100% Methanol
6. Diethyl Ether
7. dH_2O

Procedure:

1. Condition 200 mg C_{18} 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_2O.
2. Apply serum and plasma samples.
3. Wash columns with 5-10 mL dH_2O. 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 µL of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement.

Liquid-Liquid Extraction

Materials Needed:

1. 17β-Estradiol 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_2O

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

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_2O.
5. Vortex the mixture for 2 minutes, and allow phases to separate for 2 minutes.
6. Transfer organic phase to a clean glass test tube, and dry samples down using a speed vac for 2-3 hours.
7. Rehydrate samples at room temperature in the assay buffer. A minimum of 250 µL of the assay buffer is recommended for reconstitution to allow for duplicate sample measurement.

SAMPLE RECOVERIES

The 17β-Estradiol 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.

1. Wash Buffer

Prepare the wash buffer by diluting 10 mL of the supplied Wash Buffer Concentrate with 190 mL of deionized water. This can be stored at room temperature until the kit expiration, or for 3 months, whichever is earlier.

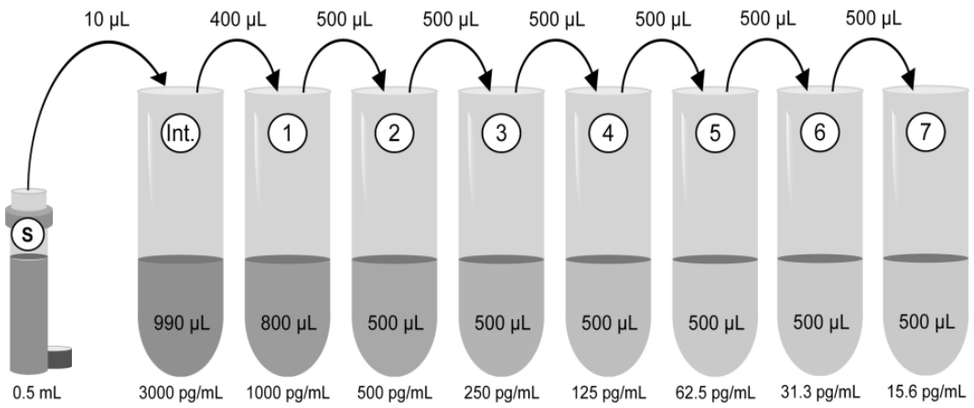
Glass or polypropylene tubes may be used for standard preparation. Avoid polystyrene.

2. 17β-Estradiol Standard

Allow the 300,000 pg/mL 17β-Estradiol standard to come to room temperature and vortex prior to use. Label seven 12 x 75 m tubes #1 through #7 and one tube "int".

Pipet 990 µL assay buffer into tube "int" and 800 µL into tube #1. Pipet 500 µL assay buffer into tubes #2 through #7.

Remove 10 µL from the stock vial and add to tube "int" and vortex thoroughly. Remove 400 µL from tube "int" and add to tube #1 and vortex thoroughly. Remove 500 µL form 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 17β-Estradiol in the tubes are labeled above.

3. 17β-Estradiol Antibody

Allow the antibody to warm to room temperature. Any unused antibody should be aliquoted and re-frozen at or below -20°C. Avoid repeated freeze-thaw cycles.

4. 17β-Estradiol Conjugate

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

5. Conjugate 1:2 Dilution for Total Activity Measurement

Prepare the Conjugate 1:2 Dilution by diluting 50 µL of the supplied Conjugate with 50 µL 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

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

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.

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

1. Pipet 150 µL of the assay buffer into the NSB (non-specific binding) wells.
2. Pipet 100 µL of the assay buffer into the Bo (0 pg/mL standard) wells.
3. Pipet 100 µL of Standards #1 through #7 to the bottom of the appropriate wells.
4. Pipet 100 µL of the samples to the bottom of the appropriate wells.

All standards and samples should be run in duplicate.

5. Pipet 50 µL of the blue conjugate into each well except the TA and Blank wells.

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

6. 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.

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 µL 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.

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.

9. Pipet 5 µL of the blue conjugate (diluted 1:2) to the TA wells.
10. Add 200 µL of the substrate solution into each well.
11. Incubate for 1 hour at room temperature without shaking.
12. Pipet 50 µL stop solution into each well.
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 dilution or concentration factor used during sample preparation.

Several options are available for the calculation of the concentration of 17β-Estradiol 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 17β-Estradiol 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.

   Average Net OD = Average 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:

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

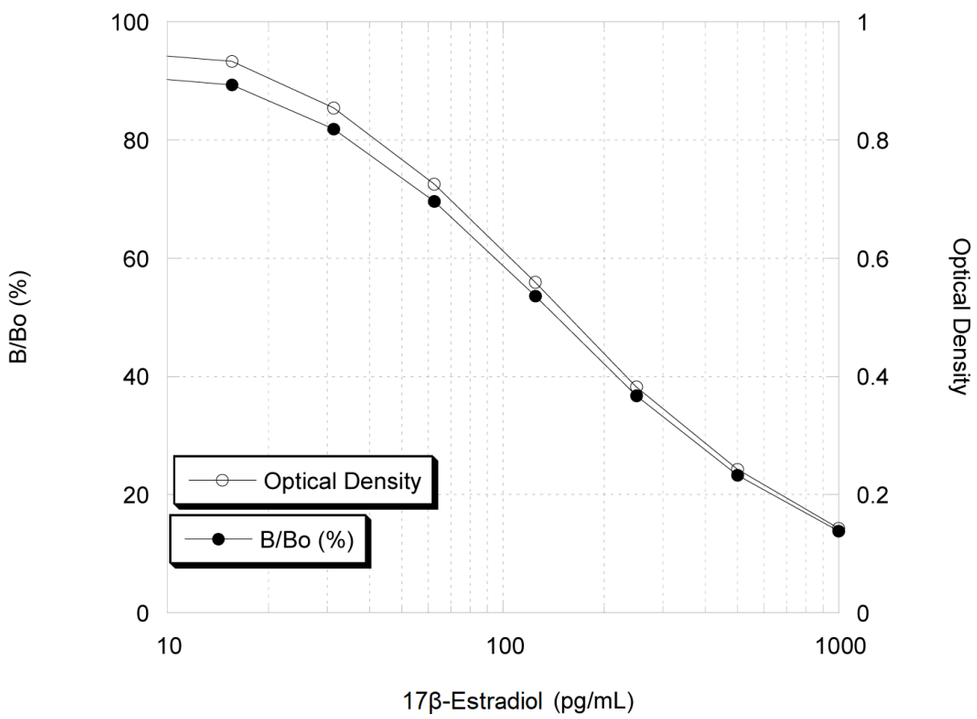
3. Plot the Percent Bound (B/Bo) versus concentration of 17β-Estradiol for the standards. Approximate a straight line through the points. The concentration of 17β-Estradiol of the unknowns can be determined by interpolation.

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	17β-Estradiol (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.

Compound	Cross Reactivity
Estrone	17.8%
Estriol	0.9%
DHT	0.12%
Ethynylestradiol-7α	0.043%
Dehydroisoandrosterone	0.038%
Estradiol-17α	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.

	Average % of Expected		
Dilution	Serum	Plasma	Pregnancy Serum
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 17β-Estradiol 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 17β-Estradiol 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.
4. I. Nemere & A.W. Norman, Molecular and Cellular Endocrinology, (1991), 80: C165.
5. E.E. Baulieu & P. Robel, “Ciba Foundation Symposium 191” (1995), John Wiley & Sons, Chichester, UK.

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com

Catalog Number: ADI-900-174
Document Number: PM-PL0116
Rev 022624