Estradiol ELISA Kit

Catalog Number KA0297

96 assays

Version: 09

Intended for research use only

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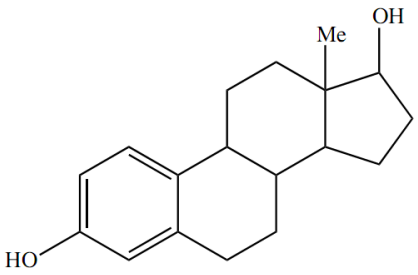
Introduction

Background

17β-Estradiol 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[3-5]. The hormone is synthesized enzymatically from acetate, cholesterol, progesterone and testosterone[3]. In addition to Estradiol's well described anatomic and physiological regulation of reproduction[4,5] and secondary sex characteristics, it also influences diverse activities such as bone growth, brain development and maturation, and the intracelluar concentration of calcium[6] and certain second messenger molecules[7].
For an extensive review of the non-reproductive actions of Estradiol see the Ciba Foundation Sym-posium, 1995.

17β-Estradiol



Principle of the Assay

17β-Estradiol kit is a competitive immunoassay for the quantitative determination of 17β-Estradiol in biological and environmental samples. Please read the complete kit insert before performing this assay. The kit for the quantitative measurement of 17β-Estradiol uses a polyclonal antibody to 17β-Estradiol to bind, in a competitive manner, 17β-Estradiol in the standard or sample or an alkaline phosphatase molecule which has 17β-Estradiol covalently attached to it. After a simultaneous incubation at room temperature 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 is read on a microplate reader at 405nm. The intensity of the bound yellow color is inversely proportional to the concentration of 17β-Estradiol in either standards or samples. The measured optical density is used to calculate the concentration of 17β-Estradiol. For further explanation of the principles and practice of immunoassays please see the excellent books by Chard[1] or Tijssen[2].

General Information

Materials Supplied

List of component

Component	Amount
Goat anti-Rabbit IgG Microtiter Plate: A plate using break-apart strips coated with goat antibody specific to rabbit IgG.	96 Wells
17β-Estradiol ELISA Conjugate: A blue solution of alkaline phosphatase conjugated with 17β-Estradiol.	5 mL
17β-Estradiol ELISA Antibody: A yellow solution of a rabbit polyclonal antibody to 17β-Estradiol.	5 mL
Assay Buffer 3: Tris buffered saline containing proteins and detergents and sodium azide as a preservative.	27 mL
Wash Buffer Concentrate: Tris buffered saline containing detergents.	27 mL
17β-Estradiol Standard: A solution of 300,000 pg/mL 17β-Estradiol.	0.5 mL
Steroid Displacement Reagent: A special formulated displacer to inhibit steroid binding to proteins.	1 mL
pNpp Substrate: A solution of p-nitrophenyl phosphate in buffer. Ready to use.	20 mL
Stop Solution: A solution of trisodium phosphate in water. Keep tightly capped. Caution: Caustic.	5 mL
Plate Sealer	1 slide

Storage Instruction

All components of this kit are stable at 4°C until the kit's expiration date.

Materials Required but Not Supplied

- ✓ Deionized or distilled water.
- ✓ Precision pipets for volumes between 5 µL and 1,000 µL.
- ✓ Repeater pipets for dispensing 50 µL and 200 µL.
- ✓ Disposable beakers for diluting buffer concentrates.
- ✓ Graduated cylinders.
- ✓ A microplate shaker.
- ✓ Adsorbent paper for blotting.
- ✓ Microplate reader capable of reading at 405 nm, preferably with correction between 570 and 590 nm.

Precautions for Use

- Precautions

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

✓ 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.

✓ Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use.

✓ The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA.

✓ 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.

✓ The 17β-Estradiol Standard provided is supplied in ethanolic buffer at a pH optimized to maintain 17β-Estradiol integrity. Care should be taken handling this material because of the known and unknown effects of steroids.

- *Procedure Notes:*

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

✓ *Allow all reagents to warm to room temperature for at least 30 minutes before opening.*

✓ *Standards can be made up in either glass or plastic tubes.*

✓ *Pre-rinse the pipet tip with reagent, use fresh pipet tips for each sample, standard and reagent.*

✓ *Pipet standards and samples to the bottom of the wells.*

✓ *Add the reagents to the side of the well to avoid contamination.*

✓ *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.*

✓ *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.*

✓ *Prior to addition of substrate, ensure that there is no residual wash buffer in wells. Any remaining wash buffer may cause variation in assay results.*

Assay Protocol

Reagent Preparation

- 17β-Estradiol Standard

  Allow the 300,000 pg/mL 17β-Estradiol standard solution to warm to room temperature. Label six 12 x 75 mm glass tubes #1 through #6. Pipet 1,000 µL of standard diluent (Assay Buffer 3 or Tissue Culture Media) into tube #1. Pipet 750 µL of standard diluent into tubes #2 through #6. Remove 100 µL of diluent from tube #1. Add 100 µL of the 300,000 pg/mL standard to tube #1. Vortex thoroughly. Add 250 µL of tube #1 to tube #2 and vortex thoroughly. Add 250 µL of tube #2 to tube #3 and vortex. Continue this for tubes #4 through #6.

  The concentration of 17β-Estradiol in tubes #1 through #6 will be 30,000, 7,500, 1,875, 468.8, 117.2 and 29.3 pg/mL respectively.

  Diluted standards should be used within 60 minutes of preparation.

- 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 date, or for 3 months, whichever is earlier.

Sample Preparation

This kit is compatible with 17β-Estradiol samples in a wide range of matrices. Many samples diluted sufficiently into Assay Buffer 3 can be read directly from the standard curve. 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. Included with the kit is the Steroid Displacement Reagent that should be added to samples containing steroid binding proteins, if samples can be measured without extraction. Samples should be diluted with 1 part of the Reagent for every 99 parts of sample prior to further dilution with Assay Buffer 3. Samples containing rabbit IgG may interfere with the assay.

Samples in the majority of Tissue Culture Media can also be read in the assay, provided the standards have been diluted into the Tissue Culture Media instead of Assay Buffer 3. There will be a small change in the binding associated with running the standards and samples in media. Users should only use standard curves generated in media or buffer to calculate concentrations of 17β-Estradiol in the appropriate matrix. Some samples may have very low levels of 17β-Estradiol present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

- Materials Needed

✓ Estradiol Standard to allow extraction efficiency to be accurately determined.

✓ ACS Grade Diethyl Ether.

✓ Glass test tubes.

- Procedure

1. Add sufficient Estradiol to a typical sample for the determination of extraction efficiency.
2. In a fume hood add 1 mL of Diethyl Ether for every 100 µL of sample. Vortex or shake the sample.
3. Allow layers to separate. Carefully pipet off the top ether layer and place in a clean test tube.
4. Repeat, combining the ether layers.
5. Evaporate the ether to dryness under nitrogen.
6. Dissolve the extracted Estradiol with Assay Buffer 3 by vortexing well then allow to sit for 5 minutes at room temperature. Repeat twice more.
7. Run the reconstituted samples in the assay immediately or keep frozen below -20°C.

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 Plate Layout 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 3 or Tissue Culture Media) into the NSB and the Bo (0 pg/mL Standard) wells.
3. Pipet 100 µL of Standards #1 through #6 into the appropriate wells.
4. Pipet 100 µL of the Samples into the appropriate wells.
5. Pipet 50 µL of Assay Buffer 3 into the NSB wells.
6. Pipet 50 µL of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipet 50 µL of 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.
8. Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided, if so desired.
9. Empty the contents of the wells and wash by adding 400 µL of wash solution to every 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 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 p-Npp Substrate solution to every well. Incubate at room temperature for 45 minutes 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, prefer-ably 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.

Data Analysis

Calculation of Results

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 this sort of data reduction software is not readily available, the concentration of 17β-Estradiol 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:

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

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 Percent Bound versus Concentration of 17β-Estradiol for the standards. Approximate a straight line through the points. The concentration of 17β-Estradiol in the unknowns can be determined by interpolation.

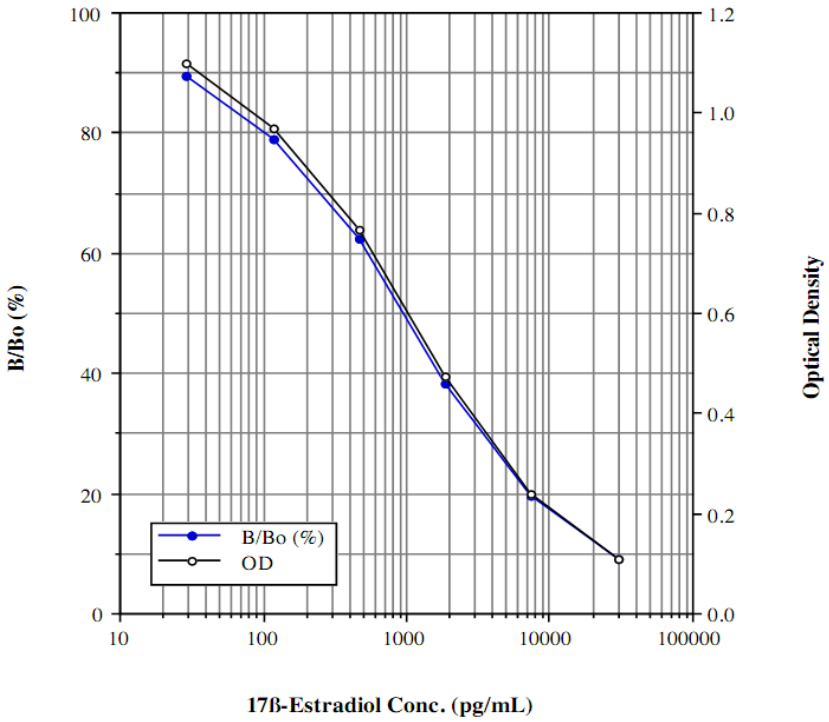
- Typical Results

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

Sample	Mean OD (-Blank)	Average Net OD	Percent Bound	17β-Estradiol (pg/mL)
Blank OD	(0.101)			
TA	0.240			
NSB	0.002	0	0.0%	
Bo	1.230	1.228	100.0%	0
S1	0.111	0.109	8.9%	30,000
S2	0.242	0.240	19.5%	7,500
S3	0.474	0.472	38.4%	1,875
S4	0.768	0.766	62.4%	468.8
S5	0.972	0.970	79.0%	117.2
S6	1.101	1.099	89.5%	29.3
Unknown 1	0.301	0.299	24.3%	4,979
Unknown 2	0.616	0.614	50.6%	955.8

- Typical Standard Curve

  A typical standard curve is shown below. This curve must not be used to calculate 17β-Estradiol concentrations; each user must run a standard curve for each assay.



- Typical Quality Control Parameters

Total Activity Added	=	0.240 x 10 = 2.40
%NSB	=	0.8%
%Bo/TA	=	51.3%
Quality of Fit	=	1.0000 (Calculated from 4 parameter logistic curve fit)
20% Intercept	=	7,196 pg/mL
50% Intercept	=	956 pg/mL
80% Intercept	=	112 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 Protocols[8].

- Sensitivity

  Sensitivity was calculated in Assay Buffer 3 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 #6. The detection limit was determined as the concentration of 17β-Estradiol measured at two

(2) standard deviations from the zero along the standard curve.

Average Optical Density for the Bo	=	1.223 ± 0.051 (4.14%)		
Average Optical Density for Standard #6	=	1.118 ± 0.042 (3.77%)		
Delta Optical Density (0-29.3 pg/mL)	=	1.223 - 1.118	=	0.105
2 SD's of the Zero Standard	=	2 x 0.051	=	0.102
Sensitivity	=	(0.102/0.105) x 29.3 pg/mL	=	28.5 pg/mL

- Linearity

  A sample containing 5,247.3 pg/mL 17β-Estradiol was diluted 6 times 1:2 in the kit Assay Buffer 3 and measured in the assay. The data was plotted graphically as actual 17β-Estradiol concentration versus measured 17β-Estradiol concentration.

  The line obtained had a slope of 0.903 and a correlation coefficient of 0.998.

- Precision

  Intra-assay precision was determined by taking samples containing low, medium and high concentrations of 17β-Estradiol and running these samples multiple times (n=16) in the same assay. Inter-assay precision was determined by measuring three samples with low, medium and high concentrations of 17β-Estradiol in multiple assays (n=8).

  The precision numbers listed below represent the percent coefficient of variation for the concentrations of 17β-Estradiol determined in these assays as calculated by a 4 parameter logistic curve fitting program.

	17β-Estradiol (pg/mL)	Intra-assay %CV	Inter-assay %CV
Low	1,327	8.4	
Medium	2,722	8.1	
High	5,094	9.2	
Low	214		7.4
Medium	1,034		6.1
High	4,959		5.2

- Cross Reactivities

  The cross reactivities for a number of related steroid compounds was determined by dissolving the cross reactant (purity checked by N.M.R. and other analytical methods) in Assay Buffer 3 at concentrations from 10,000,000 to 10 pg/mL. These samples were then measured in the 17β- Estradiol assay, and the measured 17β-Estradiol concentration at 50% B/Bo was 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
17β-Estradiol	100%
Estrone	4.64%
Estriol	0.53%
Estrone-3-Sulfate	0.36%
Progesterone	0.06%
Testosterone	0.02%
17α-Ethynylestradiol	<0.2%
19-Hydroxytestosterone	<0.02%
17α-Estradiol	<0.02%
Dehydroisoandrosterone	<0.001%
Dehydroisoandrosterone-3-sulfate	<0.001%

- Sample Recoveries

  Please refer to Sample Preparation and 17β-Estradiol Standard preparation. 17β-Estradiol concentrations were measured in a variety of different samples including tissue culture media and human saliva. 17β-Estradiol was spiked into the undiluted samples of these matrices which were then diluted with the appropriate diluent and subsequently assayed in the kit. The following results were obtained:

Sample	% Recovery*	Recommended Dilution*
Tissue Culture Media	92.2	> 1:8
Human Saliva	104.8	> 1:16

*See Sample Preparation instructions for details.

Resources

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. N.W. Tietz, “Textbook of Clinical Chemistry”, (1986) Philadelphia, PA: W.B. Saunders Co.
4. J.K. Grant & G.H. Beastall, “Clinical Biochemistry of Steroid Hormones: Methods and Applications”, (1983), Elsevier, Amsterdam.
5. M.L. Taymor, Fertility and Sterility, (1996), 65 (2): 235.
6. I. Nemere & A.W. Norman, Molecular and Cellular Endocrinology, (1991), 80: C165.
7. E.E. Baulieu & P. Robel, “Ciba Foundation Symposium 191” (1995), John Wiley & Sons, Chichester, UK.
8. National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Std1	Std5	sample	sample	sample	sample	sample	sample	sample	sample	sample
B	Blank	Std1	Std5	sample	sample	sample	sample	sample	sample	sample	sample	sample
C	TA	Std2	Std6	sample	sample	sample	sample	sample	sample	sample	sample	sample
D	TA	Std2	Std6	sample	sample	sample	sample	sample	sample	sample	sample	sample
E	NSB	Std3	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
F	NSB	Std3	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
G	B0	Std4	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample
H	B0	Std4	sample	sample	sample	sample	sample	sample	sample	sample	sample	sample