17β-Estradiol

• Intended Use

For the ultra-sensitive detection and quantitation of 17β-Estradiol in water (groundwater, surface water, well water). For other applications contact the company for application bulletins and/or specific matrix validation guidelines.

• Principle

The Abraxis 17β-Estradiol Kit applies the principles of enzyme linked immunosorbent assay (ELISA) to the determination of 17β-Estradiol. The sample to be tested is added to a disposable test tube, and pre-incubated for 30 minutes with paramagnetic particles attached with antibodies specific to 17β-Estradiol. Followed by the addition of an enzyme labeled estradiol conjugate. At this point a competitive reaction occurs between the estradiol which may be be in the sample and the enzyme labeled estradiol for the antibody binding sites on the magnetic particles. The reaction is allowed to continue for ninety (90) minutes. At the end of the incubation period, a magnetic field is applied to hold in the test tube the para-magnetic particles (with Estradiol and labeled Estradiol bound to the antibodies on the particles, in proportion to their original concentration), and allow the unbound reagents to be decanted. After decanting, the particles are washed with Washing Solution.

The presence of 17β-Estradiol is detected by adding the "Color Solution", which contains the enzyme substrate (hydrogen peroxide) and the chromogen (3,3',5,5'-tetramethylbenzidine). The enzyme-labeled Estradiol bound to the Estradiol antibody catalyzes the conversion of the substrate/ chromogen mixture to a colored product. After an incubation period, the reaction is stopped and stabilized by the addition of a diluted acid (Stopping Solution). Since the labeled Estradiol (conjugate) was in competition with the unlabeled Estradiol (sample) for the antibody sites, **the color developed is inversely proportional to the concentration of Estradiol in the sample**.

• Reagents

The Abraxis Estradiol Kit contains the following items:

1. Estradiol Antibody Coupled Paramagnetic Particles
Estradiol antibody (rabbit anti-17β-Estradiol) covalently bound to paramagnetic particles suspended in a buffered soution with preservative and stabilizers.
100 test kit: one 60 mL vial

2. Estradiol Enzyme Conjugate
Horseradish peroxidase (HRP) labeled Estradiol analog diluted in a buffered solution with preservative and stabilizers.
100 test kit: one 30 mL vial

3. 17β Estradiol Standards
Three concentrations (2.5, 7.5, 25.0 pg/mL or ppt) of 17β-Estradiol standards in distilled water with preservative and stabilizers. Each vial contains 2.0 mL.

4. Control
A concentration (approximately 10 ppt) of Estradiol in distilled water with preservative and stabilizers. A 2.0 mL volume is supplied in one vial.

5. Diluent/Zero Standard
Distilled water with preservative and stabilizers without any detectable 17β-Estradiol.
100 test kit: one 35 mL vial

6. Color Solution
A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
100 test kit: one 65 mL vial

7. Stopping Solution
A solution of diluted acid.
100 test kit: one 60 mL vial

8. Washing Solution
Preserved deionized water.
100 test kit: one 250 mL vial

9. Test Tubes
Polystyrene tubes (33) are packaged in a bag.
100 test kit: three 36 tube bags

• Reagent Storage and Stability

Store all reagents at 2-8°C. Do not freeze. Reagents may be used until the expiration date on the box. *The test tubes and Washing Solution require no special storage condition and may be stored separately from the reagents to conserve refrigerator space.*

Consult state, local and federal regulations for proper disposal of all reagents.

• Materials Required but Not Provided

In addition to the reagents provided, the following items are essential for the performance of the test:

Pipets*	Precision pipets capable of delivering 250, and 500 uL and a 1.0 mL repeating pipet.
Vortex Mixer*	Thermolyne Maxi Mix, Scientific Industries Vortex Genie, or equivalent

Magnetic Separation System*

Photometer* capable of readings at 450 nm

* Please contact Abraxis for supplier information.

• Sample Information

This procedure is recommended fo use with water samples. Other samples may require modifications to the procedure and should be thoroughly validated.

Samples containing gross particulate matter should be filtered (e.g. 0.2 um Anotop™ 25 Plus, Whatman, Inc.) to remove particles.

Samples which have been preserved with monochloroacetic acid or other acids, should be neutralized with strong base e.g. 6N NaOH, prior to assay.

If the Estradiol concentration of a sample exceeds 25 ppt, the sample is subject to repeat testing using a diluted sample. A ten-fold or greater dilution of the sample is recommended with an appropriate amount of Diluent/Zero Standard or Sample Diluent. For example, in a separate test tube make a ten-fold dilution by adding 100 uL of the sample to 900 uL of Diluent/Zero Standard. Mix thoroughly before assaying. Perform the assay according to the Assay Procedure and obtain final results by multiplying the value obtain by the dilution factor e.g. 10.

• Reagent Preparation

All reagents must be allowed to come to room temperature. The antibody coupled paramagnetic particles should be mixed thoroughly before use.

• Procedural Notes and Precautions

As with all immunoassays, a consistent technique is the key to optimal performance. To obtain the greatest precision, be sure to treat each tube in an identical manner.

Add reagents directly to the bottom of the tube while **avoiding contact between the reagents and the pipet tip**. This will help assure consistent quantities of reagent in the test mixture.

Avoid cross-contaminations and carryover of reagents by using clean pipets for each sample addition and by avoiding contact between reagent droplets on the tubes and pipet tips.

Avoid foam formation during vortexing.

The magnetic separation system consists of two parts: an upper rack which will securely hold the test tubes and a lower separator which contains the magnets used to attract the antibody coupled paramagnetic particles. During incubations the upper rack is removed from the lower separator so that the paramagnetic particles remain suspended during the incubation. **For separation steps, the rack and the separator are combined to pull the paramagnetic particles to the sides of the tubes**.

To obtain optimum assay precision, it is important to perform the separation steps carefully and consistently. Decant the rack by slowly inverting away from the operator using a smooth turning action so the liquid flows consistently along only one side of the test tube. While still inverted, place the rack on an absorbent pad and allow to drain. Lifting the rack and replacing gently onto the pad several times will ensure complete removal of the liquid from the rim of the tube. Do not bang the rack.

Mix the antibody coupled paramagnetic particles just prior to pipetting.

Do not use any reagents beyond their stated shelf life.

Avoid contact of Stopping Solution (diluted sulfuric acid) with skin and mucous membranes. If this reagent comes in contact with skin, wash with water.

• Limitations

The Abraxis 17β-Estradiol Assay will detect 17β-Estradiol specifically. Very little cross-reactivity has been observed with other hormones tested. Refer to specificity table for data on several other steroid hormones. The Abraxis Estradiol Assay kit provides screening results. As with any analytical technique (GC, HPLC, etc...) positive results requiring some action should be confirmed by an alternative method.

The total time required for pipetting the magnetic particles should be kept to two (2) minutes or less, therefore the total number of tubes that can be assayed in a run should be adjusted accordingly.

• Quality Control

A control solution at approximately 10 ppt of 17β-Estradiol is provided with the Abraxis Estradiol Assay kit. It is recommended that it be included in every run and treated in the same manner as unknown samples. Acceptable limits should be established by each laboratory.

• Assay Procedure

Read Reagent Preparation, Procedural Notes and Precautions before proceeding.

1. Label test tubes for standards, control, and samples.

Tube Number	Contents of Tube
1,2	Diluent/Zero Standard, 0 ppt
3,4	Standard 1, 2.5 ppt
5,6	Standard 2, 7.5 ppt
7,8	Standard 3, 25.0 ppt
9,10	Control
11,12	Sample 1
13, 14	Sample 2
14, 15	Sample 3

2. Add 250 uL of the appropriate standard, control, or sample.
3. Mix the Estradiol Antibody Coupled Paramagnetic Particles thoroughly and add 500 uL to each tube.
4. Vortex for 1 to 2 seconds minimizing foaming.
5. Incubate for 30 minutes at room temperature.
6. Add 250 uL of Estradiol Enzyme Conjugate to each tube.
7. Vortex for 1 to 2 seconds minimizing foam.
8. Incubate for 90 minutes at room temperature.
9. Separate in the Magnetic Separation System for **two (2) minutes**.
10. Decant and **gently** blot all tubes briefly in a consistent manner.
11. Add 1 mL of Washing Solution to each tube and allow them to remain in the magnetic separation unit for **two (2) minutes**.
12. Decant and **gently** blot all tubes briefly in a consistent manner.
13. Repeat Steps 11 and 12 an additional time.
14. Remove the rack from the separator and add 500 uL of Color Solution to each tube.
15. Vortex for 1 to 2 seconds minimizing foaming.
16. Incubate for 20 minutes at room temperature.
17. Add 500 uL of Stopping Solution to each tube.
18. Add 1 mL Washing Solution to a clean test tube. Use as blank in Step 19.
19. Read results at 450 nm within 15 minutes after adding the Stopping Solution.

• Results

Manual Calculations

1. Calculate the mean absorbance value for each of the standards.

2. Calculate the %B/Bo for each standard by dividing the mean absorbance value for the standard by the mean absorbance value for the Diluent/Zero Standard.

3. Construct a standard curve by plotting the %B/Bo for each standard on vertical logit (Y) axis versus the corresponding 17β-Estradiol concentration on horizontal logarithmic (X) axis on the graph paper provided.

4. %B/Bo for controls and samples will then yield levels in ppt of 17β-Estradiol by interpolation using the standard curve.

(Contact Abraxis for detailed application information on specific photometers.)

Photometric Analyzer

Some instrument manufacturers make available photometers allowing for calibration curves to be automatically calculated and stored. Refer to instrument operating manual for detailed instructions. To obtain results for the Abraxis Estradiol Assay on instruments allowing data tranformation the following parameter settings are recommended:

Data Reduct	:	Lin. Regression
Xformation	:	Ln/LogitB
Read Mode	:	Absorbance
Wavelength	:	450 nm
Units	:	PPB
# Rgt Blk	:	0
Calibrators:		
# of Cals	:	4
# of Reps	:	2
Concentrations:		
#1:	0.0	ppt
#2:	2.5	ppt
#3:	7.5	ppt
#4:	25.0	ppt
Range	:	1.5 - 25.0
Correlation	:	0.990
Rep. %CV	:	10%

• Expected Results

In a study with water samples from locations across the U.S., the Abraxis Estradiol Assay was shown to correlate well with a commercial clinical Estradiol immunoassay (r = 0.971).

• Performance Data

Precision

The following results were obtained:

Control	1	2	3	4
Replicates	5	5	5	5
Days	3	3	3	3
n	15	15	15	15
Mean (ppt)	2.93	4.80	10.17	22.47
% CV (within assay)	11.0	9.1	6.3	2.3
% CV (between assay)	16.1	10.7	1.8	1.4

Sensitivity

The Abraxis Estradiol Assay has an estimated minimum detectable concentration of 1.5 ppt, it was calculated by substracting 3 SD from the mean of fifty determinations of a sample known to be free of 17β-estradiol.

Recovery

Five (5) groundwater samples, were spiked with various levels of 17β-Estradiol and then assayed using the Abraxis Estradiol Assay. The following results were obtained:

Amount of 17β-Estradiol Added (ppt)	Recovery Mean (ppt)	S.D. (ppt)	%
2.5	2.06	0.39	82.4
5.0	4.33	0.53	86.7
10.0	9.65	0.92	96.5
20.0	23.19	2.20	116.0
Average			95.4

Specificity

The cross-reactivity of the Abraxis Estradiol Assay for various steroid hormones can be expressed as the dose required for 50% absorbance inhibition (50% B/Bo) divided by the 50% B/Bo concentration given by 17β-estradiol standards X 100.

Compound	Cross reactivity (%)
17β-Estradiol	100
Estrone	50
Ethynyl estradiol	1.6
Androstenedione	< 0.01
Androsterone	< 0.01
Corticosterone	< 0.01
Epiandrosterone	< 0.01
16-Epiestriol	< 0.01
Estradiol-3-H_2SO_4	0.6
17α-estradiol	0.1
Estriol	0.3
Estriol-16-glucoronide	< 0.01
Dihydroepiandrosterone	< 0.01
11-Deoxycortisol	< 0.01
11-Deoxycorticosterone	< 0.01
21-Deoxycortisol	< 0.01
Dihydrotestosterone	< 0.01
Dihydroepiandrosterone	< 0.01
20-Dihydroprogesterone	< 0.01
11-Hydroxyprogesterone	< 0.01
17α-Pregnenolone	< 0.01
17α-Progesterone	< 0.01
Pregnanediol	< 0.01
Pregnanetriol	< 0.01
Pregnenolone	< 0.01
Progesterone	< 0.01
Testosterone	< 0.01

Testing For Potential Water Interferences

Various compounds and ions spiked into water samples were tested to see if they interfered with the 17β-Estradiol ELISA. The presence of the following substances up to 20,000 PPM were found to have no significant effect in this assay: nitrate, magnesium, calcium, sulfate, phosphate. Copper and fluoride up to 2,000 PPM. Sodium Chloride up to 1M. Humic acid up to 5 PPM.

• Ordering informattion

Abraxis 17β-Estradiol Assay Kit 100T	PN 580002
Sample Diluent	PN 580003
Standard Set	PN 580004

• Assistance

For ordering or technical assistance contact:

Abraxis LLC
Sales Department
54 Steamwhistle Drive
Warminster, Pennsylvania, 18974

Phone: (215) 357-3911 * Fax: (215) 357-5232
Email: Abraxiskits.com*WEB: www.abraxiskits.com

• General Limited Warranty

Abraxis LLC warrants the products manufactured by the Company, against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**