Importance of Estradiol Determination

Estradiol (E2) is the predominant se hormone present in females. It is also presnt in males, being produced as an active metabolic product of testosterone. It represents the amjor estrogen in humans. Estardiol has not only a critical impact on reproductive and sexaul functioning, but also affects other organs including bones.

The Estradiol ELISA allows the determination of 42 samples in duplicate determination. Less than 1 mL of sample extract is required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The detection limit for Estradiol in buffer is 5 pg/mL; in milk (after correcting for the 1:50 dilutiuon) is 350 pg/mL (mean of 6 blank determinations minus 3 standard deviations). The middle of the test (50% B/B_0) is at approximately 25 pg/mL. Determinations closer to the middle of the calibration curve give the most accurate results. Lower detection limits are possible.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Specificity: The cross-reactivity of the Abraxis Estradiol Kit for various other estrogens is as follows:

17-ß Estradiol	100%
Estrone	50%
Ethynyl Estradiol	1.6%
Other tested	<1%

Recoveries: Recoveries of Estradiol from spiked whole milk are between 80-120%.

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**

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Estradiol (E2) ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Estradiol in Milk Samples

Product No. 580003

1. General Description

The Estradiol ELISA is an immunoassay for the quantitative and sensitive screening of Estradiol. This test is suitable for the quantitative and/or qualitative screening of Estradiol in milk and milk products (please refer to the appropriate technical bulletins for extraction/dilution procedures). Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions of the test kit contain small amounts of Estradiol. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The Estradiol ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of Estradiol (E2) by specific antibodies. Estradiol, when present in a sample, and an E2-enzyme conjugate compete for the binding sites of anti-Estradiol antibodies in solution. The Estradiol antibodies are then bound by a second antibody (goat anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of Estradiol present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the Estradiol ELISA, Possible Test Interference

Although many organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can't be completely excluded. Milk fats will cause interference in the test, therefore milk samples should be diluted as instructed in the sample preparation step (Section H) before testing in the ELISA.

Mistakes in handling the test also can cause errors. Possible sources for such errors can be: inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme outside temperatures (lower than 10 °C or higher than 30 °C) during the test performance.

The Abraxis Estradiol ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring some regulatory action should be confirmed by an alternative method.

A. Reagents and Materials Provided

1. Microtiter plate (12 X 8 strips) coated with polyclonal anti-Estradiol antibody, in an resealable aluminum pouch
2. Calibrators/Standards (6): 0, 7.5, 15, 25, 50, 100 pg/mL (ppt) of Estradiol, 1 mL each
3. Estradiol-HRP Conjugate, 6 mL
5. Estradiol Antibody Solution, 6 mL
5. Wash Solution (5X) Concentrate, 100 mL. Must be diluted 1:5 with deionized water before use
6. Substrate (Color) Solution (TMB), 16 mL
7. Stop Solution, 12 mL (Handle with care)

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, the substrate solution and the stop solution in order to equalize the incubation periods on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the aluminum pouch. The remaining strips are stored in the aluminum pouch and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
4. Dilute the wash buffer at a ratio of 1:5. If using the entire bottle (100 mL), add to 400 mL of deionized or distilled water.
5. The stop solution must be handled with care as it contains diluted H_2SO_4.

C. Assay Procedure

1. Add 25 µL of the **standard solutions** or the **diluted sample** (Section H) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of **enzyme conjugate** solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 50 µL of **antibody solution** to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 90 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips **four times** using the 1X washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 150 µL of **substrate (color) solution** to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 20-30 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of **stop solution** to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs such as Logit/Log or 4-Parameter (preferred). For a manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding Estradiol concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppt or pg/mL of Estradiol by interpolation using the standard curve. Results can also be obtained by using a spreadsheet macro available from Abraxis upon request.

The concentrations of the samples are determined using the standard curve run with each test. The concentration of Estradiol in the milk sample is determined by multiplying the ELISA concentration obtained in the assay by 50 to account for the dilution factor. Samples showing a lower concentrations of Estradiol compared to standard 1 (7.5 pg/mL or ppt) must be reported as containing < 350 ppt Estradiol. Samples showing a higher concentration than standard 5 (100 pg/mL) must be diluted further to obtain accurate results.

Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators. Sample containing less color than a calibrator will have a concentration of Estradiol greater than the concentration of the calibrator. Samples containing more color than a calibrator will have a concentration less than the concentration of the calibrator.

E. Additional Materials (not delivered with the test kit)

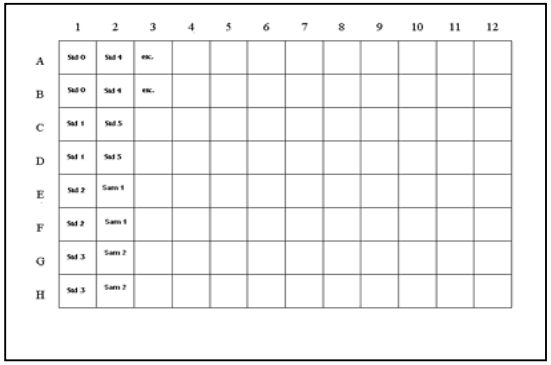
1. Micro-pipettes with disposable plastic tips (25-200 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wave length 450 nm)
5. Shaker for microtiter plates (optional)
6. Deionized or distilled water
7. Paper towels or equivalent absorbent material
8. Timer
9. Dilution Test tubes

F. Working Scheme

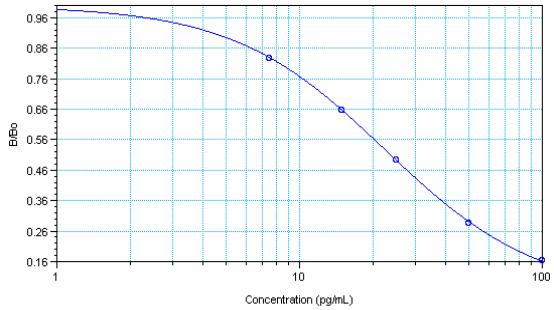
The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 5: Standards
(0; 7.5; 15; 25; 50; 100 ppt)

Sam1, Sam2, etc.: Samples



G. Standard Curve (These values are used for demonstration purposes; do not use these values for your determinations)



H. Liquid Milk Sample Dilution (1:50)

1. Pipette 980 uL of 1X PBS buffer into a test tube.
2. Pipette 20 uL of milk into the Test tube, carefully vortex or shake well to mix
3. Run the middle sample directly in the assay (step 1 on Assay Procedure).
4. Highly contaminated samples (samples outside the standard curve range) should be diluted in order to get the value in the middle of the curve and re-analyzed.

The concentration of Estradiol in the milk sample is determined by multiplying the ELISA concentration obtained in the assay by 50 to account for the dilution factor.
NOTE: Powder milk samples should be reconstituted according to manufacturer's instructions and then diluted 1:50 in 1X PBS before testing in the assay.