ELISA Kit

Catalog # KAQ0621 (96 tests)

Human
Estradiol (E2)

www.invitrogen.com
Invitrogen Corporation
542 Flynn Road, Camarillo, CA 93012
Tel: 800-955-6288
E-mail: techsupport@invitrogen.com

Table of Contents

Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	Quantity	Color Code
Standard 0 pg/mL in human serum, with preservatives. Lyophilized. Refer to vial label for quantity and reconstitution volume.	1 vial	Yellow
Standards 1 - 5 in human serum, with preservatives. Lyophilized. Refer to vial label for concentration and reconstitution volume.	5 vials	Yellow
Controls 1 and 2 in human serum, with preservatives. Lyophilized. Refer to vial label for reconstitution volume and range.	2 vials	Silver
Anti-Rabbit IgG-Coated Wells, 96 wells per plate.	1 plate	Blue
Anti-Estradiol; lyophilized. Refer to vial label for reconstitution volume.	1 vial	Blue
Estradiol-HRP Conjugate Concentrate. Contains phospate buffer with preservatives; 0.5 ml per vial.	1 vial	Red
Conjugate Buffer for dilution of Estradiol-HRP conjugate; 6 mL per vial	3 vials	Red
Wash Buffer Concentrate (200x). 10 mL per bottle.	1 bottle	Brown
Concentrated Chromogen, *Tetramethylbenzidine (TMB)* in DMF, 1 mL per vial	1 vial	Green
*Substrate Buffe*r: H_2O_2 in acetate/citrate buffer; 21 mL per bottle	3 bottles	White
Stop Solution; 1.8N H_2SO_4; 6 mL per bottle	1 bottle	Black
Note: Standard 0 pg/mL is recommended for sample dilutions.		

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Avoid any skin contact with H_2SO_4, H_2O_2 and TMB. In case of contact, wash thoroughly with water.

Introduction

Purpose

The Invitrogen Estradiol (E2) ELISA is a competitive binding immunoassay for the quantitative determination of 17-beta –estradiol (E2) in serum and plasma.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Estradiol kit is a solid phase Enzyme Linked-Immuno-Sorbent Assay (ELISA). A fixed amount of estradiol labeled with horseradish peroxidase (HRP) competes with unlabeled estradiol present in standards or samples for a limited number of binding sites of a specific antibody. The E2-HRP-antibody complex is simultaneously fixed on the wells of the microtiter plate coated with an excess of anti-rabbit-gammaglobulins.

Neither extraction nor chromatography is required due to the high specificity of the antibody.

After 2 hours incubation at room temperature the microtiter plate is washed to stop the competition reaction. The chromogen solution tetramethylbenzidine (TMB in substrate buffer) is added and incubated for 30 minutes. The reaction is stopped with H_2SO_4 and the absorbance is measured at the appropriate wavelength. The intensity of this colored product is **inversely** proportional to the concentration of E2 present in the original specimen.

Background Information

17-beta-estradiol (E2) is a C-18 steroid hormone (molecular weight 272.4) produced mainly by the ovary and placenta, and in small amounts by adrenals and testes. Estradiol is in equilibrium with estrone, which can be converted to estriol by the liver and placenta.

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
11. In-house controls or kit controls, if provided, should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with the *Wash Buffer Concentrate (200x)* provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 ml of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Samples and Reagents

Sample Preparation

No special pretreatment of the sample is necessary. Prior to use, all the samples should be at room temperature. It's recommended to vortex the samples before use.

Do not use hemolyzed samples.

Samples may be stored for up to 72 hours at 2 to 8°C prior to testing. Samples held for longer time should be frozen at -20°C prior to assaying.

Standards and Controls

Reconstitute the lyophilized Standards and Controls to the volume specified on the vial label with distilled water (4 mL for the zero standard and 0.5 mL for the other standards and controls). Allow them to remain undisturbed until completely dissolved, and then mix well by gentle inversion. See vial label for exact concentration.

After reconstitution, store the standards and controls at 2 to 8°C for 1 week maximum. For prolonged storage they must be frozen. Avoid freeze-thaw cycles.

Anti-Estradiol

Reconstitute the lyophilized Anti-Estradiol Antibody to the volume specified on the vial label (6 mL distilled water). Allow it to remain undisturbed until completely dissolved, then mix well by gentle inversion.

After reconstitution, store the Anti-Estradiol at 2 to 8°C for 1 week maximum. For prolonged storage it must be frozen. Avoid freeze-thaw cycles.

Estradiol-HRP Conjugate

Pipette 0.1 mL of the concentrated HRP-estradiol solution into one of the vials of conjugate buffer. Prepare immediately prior to use.

Maximum stability is 4 hours at room temperature or 24 hours at 2 to 8°C when protected from direct exposure to sunlight.

Wash Buffer

Dilute 2 mL of Washing Solution Concentrate in 400 mL distilled water or all the contents of the Wash Solution Concentrate vial in 2000 mL distilled water (use a magnetic stirrer).

The Wash Solution Concentrate is stable at room temperature until expiration date. In order to avoid washerhead obstructions, it is recommended to prepare a fresh diluted Wash Solution each day.

Chromogen Solution

Pipette 0.2 mL of the Concentrated Chromogen (TMB) into one of the vials of Substrate Buffer (H_2O_2 in acetate/citrate buffer). Prepare immediately prior to use. Use only at room temperature. Avoid direct exposure to sunlight.

The freshly prepared Chromogen Solution is stable for a maximum of 15 min at room temperature and must be discarded afterwards.

Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame for current use. (Re-bag extra strips. Store these in the refrigerator for future use.)
2. Dispense 50 µL of each standard, control or sample into the appropriate wells. Vertical alignment is recommended.
3. Dispense 50 µL of estradiol-HRP conjugate into all wells.
4. Dispense 50 µL of anti-estradiol into each well.
5. Incubate for **2 hours at room temperature** on a horizontal shaker set at 700 ± 100 RPM.
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Add 200 µL of *Chromogen solution* to each well. The liquid in the wells will begin to turn blue.
8. Incubate for **30 minutes at room temperature and in the dark**. ***Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceeds the limits of the instrument.
9. Add 50 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
10. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µl each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 1 hour after adding the *Stop Solution*.
11. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
12. Read the concentrations for unknown samples and controls from the standard curve.
13. For each standard and sample calculate the percent bound:

$$B/Bo \times 100 = \frac{OD\ (standard\ or\ sample)}{OD\ (zero\ standard)} \times 100$$

14. By interpolation of the samples (B/Bo x 100) values, determine the estradiol concentrations of the samples from the reference curve.

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 935 pg/mL E2.

Standard E2 pg/mL	Optical Density (450 nm)	B/Bo x 100
935	0.221	12
270	0.447	25
100	0.762	43
50	1.013	57
13	1.424	80
0	1.790	100

Performance Characteristics

Analytical Sensitivity

Minimum detectable concentration (MDC) of estradiol in 10 different assays was 5 ± 2 pg/mL (mean ± SD). MDC is defined as the concentration of estradiol corresponding to 95% of maximum binding.

Precision

1. Intra-Assay Precision

Samples of known Hu Estradiol concentration were assayed in replicates of 20 to determine precision within an assay.

	Sample 1	Sample 2
Mean (pg/ml)	131	257
SD	6	10
%CV	4.6	3.9
SD = Standard Deviation CV = Coefficient of Variation		

2. Inter-Assay Precision

Samples were assayed 15 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2
Mean (pg/ml)	101	196
SD	6	12
%CV	6.0	6.1
SD = Standard Deviation CV = Coefficient of Variation		

Linearity of Dilution

Serum dilution	Theoretical conc. (pg/mL)	Measured conc. (pg/mL)	Recovery (%)
1/1	997	997	100
1/2	498	485	97
1/4	249	252	101
1/8	125	109 87	87

Recovery

Sample	Added (pg/mL)	Recovery (pg/mL)	Recovery (%)
Serum	916	719	78.4
	516	430	83.3
	316	304	96.2
	166	176	106.0

Antigenic Specificity

The percentage of cross-reaction was estimated under physiological conditions in serum by comparison of the concentration yielding a 50% binding inhibition:

Substances	Cross-reactivity (%)
17-β estradiol	100
Estrone	2
Estriol	1.9
E2-3-Glucuronide	0.6
E2-17-Glucoronide	0.56
E2-17-Valerate	0.1
Cartisol	<0.001
Progesterone	0.03
Dhea-sulfate	<0.0001
Testosterone	<0.001
Androstenediol	<0.001
Norgestrel	0.01
Premarin	0.06
Equilin	0.1

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Estradiol in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background	*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing HRP-Conjugate can elevate the background if residual is left in the well. *Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain, tapping forcefully if necessary to remove residual fluid. *Cause:* Contamination of substrate solution with metal ions or oxidizing reagents. *Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. *Cause:* Contamination of pipette, dispensing reservoir or substrate solution with HRP Conjugate. *Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. *Cause:* Incubation time is too long or incubation temperature is too high. *Solution:* Reduce incubation time and/or temperature.
Elevated sample/ standard ODs	*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly. *Solution*: Follow the protocol instructions regarding the dilution of the standard. *Cause:* Incorrect dilution of the HRP Conjugate. *Solution:* Warm solution of HRP Conjugate concentrate to room temperature, draw up slowly and wipe tip with laboratory wipe to remove excess. Dilute ONLY in HRP diluent provided. *Cause*: Incubation times extended. *Solution:* Follow incubation times outlined in protocol. *Cause:* Incubations carried out at 37°C when RT is dictated. *Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.
Poor standard curve	*Cause:* Improper preparation of standard stock solution. *Solution:* Dilute lyophilized standard as directed by the vial label only with the recommended diluent buffer or in a diluent that most closely matches the matrix of your sample. *Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. *Solution:* NEVER substitute any components from another kit. *Cause*: Errors in pipetting the standard or subsequent steps. *Solution*: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops	*Cause:* Reagents not at RT (25 ± 2°C) at start of assay. *Solution:* Allow ALL reagents to warm to RT prior to commencing assay. *Cause:* Incorrect storage of components, e.g., not stored at 2 to 8°C. *Solution:* Store all components exactly as directed in protocol and on labels. *Cause:* Working HRP Conjugate solution made up longer than 15 minutes before use in assay. *Solution:* Use the diluted HRP Conjugate within 15 minutes of dilution. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions. *Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. *Solution:* Please contact Technical Support for advice when using alternative sample types. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.
Poor Precision	*Cause:* Errors in pipetting the standards, samples or subsequent steps. *Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. *Cause:* Repetitive use of tips for several samples or different reagents. *Solution:* Use fresh tips for each sample or reagent transfer. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

USA:

Invitrogen Corporation
542 Flynn Road
Camarillo, CA 93012

Tel: 800-955-6288

E-mail: techsupport@invitrogen.com

Europe:

Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK

Tel: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6117

E-mail: eurotech@invitrogen.com

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, please contact our Technical Support Representatives. Invitrogen warrants that all of its products will perform according to the specifications stated on the Certificate of Analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

Licensing Information

These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Estradiol Assay Summary

SUMMARY OF ASSAY PROCEDURE		
	Standards (mL)	Controls or samples (mL)
Standards	50	-
Controls-samples	-	50
Estradiol-HRP	50	50
Anti-estradiol	50	50
Incubate for 2 hours at RT with continuous shaking (700 RPM) Aspirate the content of each well Wash 5 times with 0.4 mL of wash solution and aspirate		
Substrate solution	200	200
Incubate 30 minutes at RT with continuous shaking (700 RPM)		
H_2SO_4	50	50
Read the microtiter plate 450 nm (versus chromogen blank)		