



ARCHITECT

SYSTEM

en

Estradiol

REF 7K72

48-9132/R3

B7K720


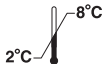


Read Highlighted Changes
Revised November, 2009

Estradiol

Customer Service: Contact your local representative or find country specific contact information on www.abbottdiagnostics.com

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF	List Number	CAL A	Calibrator (A-F)
IVD	<i>In Vitro</i> Diagnostic Medical Device	CONTROL L	Control Low, Medium, High (L, M, H)
LOT	Lot Number	REACTION VESSELS	Reaction Vessels
	Expiration Date	SAMPLE CUPS	Sample Cups
	Store at 2-8°C	SEPTUM	Septum
	Consult instructions for use	REPLACEMENT CAPS	Replacement Caps
	Manufacturer	REAGENT LOT	Reagent Lot
		SN	Serial Number
		WARNING: SENSITIZER	Warning: May cause an allergic reaction

See **REAGENTS** section for a full explanation of symbols used in reagent component naming.

NAME

ARCHITECT Estradiol

INTENDED USE

The ARCHITECT Estradiol assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of estradiol in human serum and plasma.

SUMMARY AND EXPLANATION OF TEST

Estradiol is the most potent natural estrogen in humans. It regulates reproductive function in females, and, with progesterone, maintains pregnancy. Most estradiol is secreted by the ovaries (non-pregnant women), although the testes (in men) and adrenal cortex (in men and women) secrete small amounts. During pregnancy, the placenta produces most of the circulating estradiol.

Estradiol and estrone interconvert *in vivo*. In normal non-pregnant women, estradiol synthesized by the ovary is the predominant source of both estrone and estradiol.

Virtually all circulating estradiol is protein-bound. Reported association constants for estradiol with sex hormone binding globulin and serum albumin are, respectively, 6.8×10^8 and 6×10^4 .¹ One consequence of this binding is that the conditions of any assay for serum estradiol must release this steroid quantitatively from its binding partners. The amount and proportion of protein-bound and free estradiol vary by gender, and with pregnancy and menstrual phase in women.¹

Normal estradiol levels are lowest at menses and into the early follicular phase (25-75 pg/mL) and then rise in the late follicular phase to a peak of 200-600 pg/mL just before the LH surge, which is normally followed immediately by ovulation. As LH peaks, estradiol begins to decrease before rising again during the luteal phase (100-300 pg/mL). If conception does not take place, estradiol falls further to its lowest levels, and menses begins shortly thereafter.²⁻⁵ If conception occurs, estradiol levels continue to rise, reaching levels of 1,000-5,000 pg/mL during the first trimester, 5,000-15,000 pg/mL during second trimester, and 10,000-40,000 pg/mL during third trimester.⁶⁻⁸ At menopause, estradiol levels remain low.²

Because the ovaries produce most estradiol in normal women, estimation of this hormone is sometimes a gauge of ovarian function.⁹ In addition, monitoring estradiol levels is important in evaluating amenorrhea, precocious puberty, the onset of menopause, and infertility in men and women. Monitoring estradiol levels is essential during *in vitro* fertilization, because the timing of recovery of oocytes depends on follicular development, which in turn depends on the estradiol level.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The ARCHITECT Estradiol assay is a delayed one step immunoassay to determine the presence of estradiol in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.

In the first step, sample, specimen diluent, assay diluent, and anti-estradiol (rabbit, monoclonal) coated paramagnetic microparticles are combined. Estradiol present in the sample binds to the anti-estradiol coated microparticles. After an incubation, estradiol acridinium labeled conjugate is added to the reaction mixture. After a second incubation, and washing, Pre-Trigger and Trigger Solutions are then added and the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of estradiol in the sample and the RLUs detected by the ARCHITECT *i* optical system.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Reagent Kit, 100 Tests

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT *i* Systems. Please contact your local distributor.

ARCHITECT Estradiol Reagent Kit (7K72)

- **MICROPARTICLES** 1 or 4 Bottle(s) (9.9 mL) Anti-Estradiol (rabbit, monoclonal) coated Microparticles in TRIS/BIS-TRIS buffer with protein (rabbit) stabilizers. Minimum Concentration: 0.0657% solids. Preservative: ProClin.
- **CONJUGATE** 1 or 4 Bottle(s) (5.9 mL) Estradiol acridinium-labeled Conjugate in citrate buffer with surfactant stabilizers. Minimum concentration: 63.36 ng/mL. Preservative: ProClin.
- **ASSAY DILUENT** 1 or 4 Bottle(s) (5.9 mL) Estradiol Assay Diluent containing surfactant in citrate buffer. Preservative: ProClin.
- **SPECIMEN DILUENT** 1 or 4 Bottle(s) (10.0 mL) Estradiol Specimen Diluent containing TRIS buffer with protein (bovine) stabilizers. Preservative: Sodium Azide.

Other Reagents

ARCHITECT *i* Pre-Trigger Solution

- **PRE-TRIGGER SOLUTION** Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

ARCHITECT *i* Trigger Solution

- **TRIGGER SOLUTION** Trigger Solution containing 0.35N sodium hydroxide.

ARCHITECT *i* Wash Buffer

NOTE: Bottle and volume varies based on order.

- **WASH BUFFER** Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

WARNINGS AND PRECAUTIONS

- **IVD**
- For *In Vitro* Diagnostic Use
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens.¹⁰ Biosafety Level 2¹¹ or other appropriate biosafety practices^{12,13} should be used for materials that contain or are suspected of containing infectious agents.

- The ARCHITECT Estradiol Microparticles, Conjugate and Assay Diluent contain methylisothiazolones which are components of ProClin, and are classified per applicable European Community (EC) directives as: Irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.



- | | |
|-----|---|
| R43 | May cause sensitization by skin contact. |
| S24 | Avoid contact with skin. |
| S35 | This material and its container must be disposed of in a safe way. |
| S37 | Wear suitable gloves. |
| S46 | If swallowed, seek medical advice immediately and show this container or label. |

- This product contains sodium azide; for a specific listing, refer to the **REAGENTS** section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

- For information on the safe disposal of sodium azide and a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.


Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the ARCHITECT Estradiol Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment. For microparticle mixing instructions, refer to the **Mixing Instructions** in this package insert.
- Septums **MUST** be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.
- **ARCHITECT Estradiol requires the use of List Number 4D18-02 or higher septums.**
- To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
- Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results.
- Over time, residual liquids may dry on the septum surface. These are typically dried salts which have no effect on assay efficacy.
- For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Mixing Instructions

- Before loading the ARCHITECT Estradiol Reagent Kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that have settled during shipment:
 - Invert the microparticle bottle 30 times.
 - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
 - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
 - Once the microparticles have been resuspended, remove and discard the cap. Wearing clean gloves, remove a septum from the bag. Carefully snap the septum onto the top of the bottle.
- ARCHITECT Estradiol Calibrators and Controls should be mixed by gentle inversion prior to use.

Storage Instructions

-  The ARCHITECT Estradiol Reagent Kit must be stored at 2-8°C and may be used immediately after removal from 2-8°C storage.
- When stored and handled as directed, reagents are stable until the expiration date.
- The ARCHITECT Estradiol Reagent Kit may be stored on-board the ARCHITECT *i* System for a maximum of 30 days. After 30 days, the reagent kit must be discarded. For information on tracking on-board time, refer to the ARCHITECT System Operations Manual, Section 5.
- Reagents may be stored on or off the ARCHITECT *i* System. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, place them in their original trays and boxes to ensure they remain upright and protect from long-term exposure to light. If any reagent bottle does not remain upright (with a septum installed) while in 2-8°C storage off the system, the reagent kit must be discarded. After reagents are removed from the system, initiate a scan to update the on-board stability timer.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results may be invalid and may require retesting. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

- **The ARCHITECT Estradiol assay requires that ARCHITECT *i* Trigger Solution be stored on-board for no longer than 10 days after the day it is installed. Write the on-board expiration date on the trigger bottle (install date plus 10 days is the on-board expiration date).**

NOTE: Trigger can be used through the on-board expiration date. This date must not exceed the printed expiration date of the ARCHITECT *i* Trigger Solution.

- Install the **_Estradiol** assay file from the ARCHITECT *i* Assay CD-ROM on the ARCHITECT *i* System before performing this Estradiol assay. For detailed information on assay file installation and on viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.
- For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.
- For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.
- The default result unit for the ARCHITECT Estradiol assay is pg/mL. For information regarding alternate result units see the **RESULTS** section of this package insert.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

- Human serum (including serum collected in serum separator tubes) or plasma collected in lithium heparin (including plasma separator tubes) or potassium EDTA collected in glass or plastic may be used in the ARCHITECT Estradiol assay. Other anticoagulants have not been validated for use with the ARCHITECT Estradiol assay.

- Refer to the specimen collection tube manufacturer's instructions as well as these package insert instructions for specimen collection and preparation for analysis. Further specimen handling information can be found in **Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) Guideline H18-A2.**¹⁴
- The ARCHITECT *i* System does not provide the capability to verify specimen type. It is the responsibility of the operator to verify the correct specimen types are used in the ARCHITECT Estradiol assay.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- For optimal results, inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- If testing will be delayed more than 24 hours, remove serum or plasma from the clot, separator, or red blood cells and store at 2-8°C. Specimens may be stored for up to 7 days at 2-8°C prior to being tested. If testing will be delayed more than 7 days, specimens should be frozen at -20°C or colder.¹⁵
- Multiple freeze-thaw cycles of specimens should be avoided. Specimens must be mixed **THOROUGHLY** after thawing, by LOW speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Sample from the middle of the tube to avoid any particulates on the top or bottom of the specimen.
- Do not use heat-inactivated specimens.
- Performance has not been established using cadaver specimens or body fluids other than human serum or plasma.
- Specimens with obvious microbial contamination should not be used.
- When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Prior to shipment, it is recommended that specimens be removed from the clot, separator, or red blood cells.

Sample Volume

- The minimum sample cup volume is calculated by the system and is printed on the Orderlist report. No more than 9 replicates may be sampled from the same sample cup. To minimize the effects of evaporation verify adequate sample cup volume is present prior to running the test.
 - Priority: 200 µL for the first Estradiol test plus 150 µL for each additional Estradiol test from the same sample cup
 - ≤ 3 hours on-board: 200 µL for the first Estradiol test plus 150 µL for each additional Estradiol test from the same sample cup
 - > 3 hours on-board: additional sample volume is required. Refer to the ARCHITECT System Operations Manual, Section 5 for information on sample evaporation and volumes.
- If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.

PROCEDURE

Materials Provided

- 7K72 ARCHITECT Estradiol Reagent Kit

Materials Required but not Provided

- ARCHITECT *i* System
- ARCHITECT *i* Assay CD-ROM
- 7K72-01 ARCHITECT Estradiol Calibrators
- 7K72-10 ARCHITECT Estradiol Controls
- 7K72-50 ARCHITECT Estradiol Manual Diluent
- ARCHITECT *i* **PRE-TRIGGER SOLUTION**
- ARCHITECT *i* **TRIGGER SOLUTION**

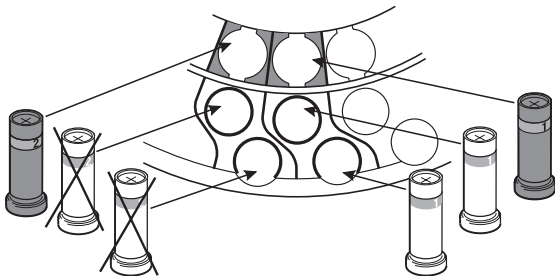
- ARCHITECT *i* **WASH BUFFER**
- ARCHITECT *i* **REACTION VESSELS**
- ARCHITECT *i* **SAMPLE CUPS**
- ARCHITECT *i* **SEPTUM** List Number 4D18-02 or higher
- ARCHITECT *i* **REPLACEMENT CAPS**
- For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

Assay Procedure

- The ARCHITECT Estradiol assay requires that ARCHITECT *i* Trigger Solution be stored on-board for no longer than 10 days after the day it is installed. Write the on-board expiration date on the trigger bottle (install date plus 10 days is the on-board expiration date).

NOTE: Trigger can be used through the on-board expiration date. This date must not exceed the printed expiration date of the ARCHITECT *i* Trigger Solution.

- Order tests.
- Load the ARCHITECT Estradiol Reagent Kit on the ARCHITECT *i* System. Verify that all necessary assay reagents are present. Ensure that septums are present on all reagent bottles.
 - The reagent carousel has color coded rings which match the color bands on the reagent bottle labels.
 - The color bands on the reagent bottle labels have a #1 or #2 designation.
 - The diagram on the inside of the reagent carousel cover and the following diagram can be used to facilitate reagent loading.



- Place the bottle with the yellow color band #1 (Assay Diluent) in the yellow ring of the carousel.
- Place the bottle with the pink color band #1 (Microparticles) in the pink ring of the carousel.
- Place the bottle with the green color band #1 (Specimen Diluent) in the green ring of the carousel.
- Place the bottle with the yellow color band #2 (Conjugate) in the yellow ring of the carousel to the **left** of the bottle with the #1 designation.
- **NOTE:** Estradiol Reagent Kits do not contain bottles with a green and pink #2 designation.
- Do not place Estradiol reagent bottles with a #1 designation in reagent carousel position 25 and reagent bottles with a #2 designation in reagent carousel position 1.
- ARCHITECT Estradiol Calibrators and Controls should be mixed by gentle inversion prior to use.
 - To obtain the recommended volume requirements for the ARCHITECT Estradiol Calibrators and Controls, hold the bottles vertically and dispense 15 drops (400 μ L) of each calibrator or 10 drops (250 μ L) of each control into each respective sample cup.
 - After each use tightly close the caps, place bottles in carton to protect from light and return the calibrators and controls to 2-8°C storage.
- Load samples.
 - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN. The ARCHITECT *i* System performs the following functions:
 - Moves the sample to the aspiration point
 - Loads a reaction vessel (RV) into the process path

- Aspirates and transfers sample into the RV
- Advances the RV one position and transfers specimen diluent, microparticles, and assay diluent into the RV
- Mixes and incubates the reaction mixture
- Adds conjugate to the RV
- Mixes, incubates and washes the reaction mixture
- Adds Pre-Trigger and Trigger Solutions
- Measures chemiluminescent emission to determine the quantity of estradiol in the sample
- Aspirates contents of RV to liquid waste and unloads RV to solid waste
- Calculates the result
- For information on ordering patient specimens, calibrators and controls, and general operating procedures refer to the ARCHITECT System Operations Manual, Section 5.
- For optimal performance, it is important to follow the routine maintenance procedures defined in the ARCHITECT System Operations Manual, Section 9. If your laboratory requires more frequent maintenance, follow those procedures.

Specimen Dilution Procedures

Specimens with an estradiol value exceeding 1,000 pg/mL are flagged with the code “ >1000” and may be diluted with the Automated Dilution Protocol.

- If using the Automated Dilution Protocol, the system performs a 1:5 dilution of the specimen and automatically calculates the concentration of the undiluted specimen and reports the result.
- Specimens with an estradiol value exceeding 5,000 pg/mL are flagged with the code “ >5000” when run using the Automated Dilution Protocol. These specimens may be diluted with the Manual Dilution Protocol.
- Manual dilutions should be performed as follows:
 - The suggested dilution for estradiol is 1:10.
 - For example, add 20 μ L of the patient specimen to 180 μ L of ARCHITECT Estradiol Manual Diluent.
 - The operator must enter the dilution factor in the patient or control order screen. The system will use this dilution factor to automatically calculate the concentration of the specimen before dilution. This will be the reported result. The concentration of the diluted specimen (before dilution factor is applied) should be greater than 100 pg/mL.
- For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- To perform an ARCHITECT Estradiol calibration, test Calibrators A through F in duplicate. A single sample of all levels of estradiol controls must be tested to evaluate the assay calibration. Ensure that assay control values are within the concentration ranges specified in the control package insert. Calibrators should be priority loaded.
- Calibration Range: 0 - 1000 pg/mL.
- Once an ARCHITECT Estradiol calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
 - A reagent kit with a new lot number is used
 - Controls are out of range
- For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

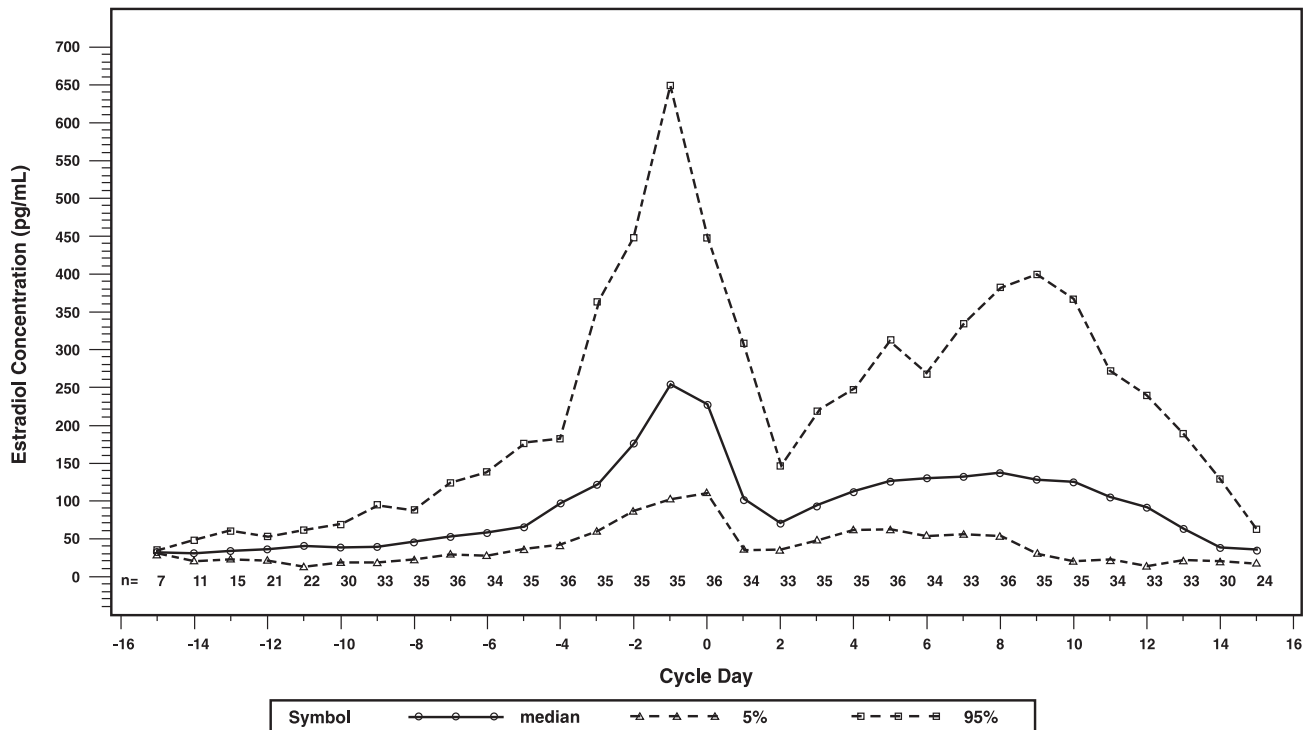
QUALITY CONTROL PROCEDURES

The recommended control requirement for the ARCHITECT Estradiol assay is a single sample of all control levels tested once every 24 hours each day of use. If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures. Ensure that assay control values are within the concentration ranges specified in the control package insert.

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B. The ARCHITECT Estradiol assay belongs to method group 1.

ARCHITECT Estradiol Profile During the Normal Menstrual Cycle



RESULTS

The ARCHITECT Estradiol assay utilizes a 4 Parameter Logistic Curve Fit data reduction method (4PLC, Y weighted) to generate a calibration curve.

Alternate Result Units

- The default result unit for the ARCHITECT Estradiol assay is pg/mL. When the alternate result unit, pmol/L, is selected, the conversion factor used by the system is 3.67. When the alternate result unit, nmol/L, is selected, the conversion factor used by the system is 0.00367.
- Conversion Formula: (Concentration in pg/mL) x (3.67) = pmol/L
- Conversion Formula: (Concentration in pg/mL) x (0.00367) = nmol/L

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the ARCHITECT System Operations Manual, Section 5.

LIMITATIONS OF THE PROCEDURE

- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- If the estradiol results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.¹⁶ Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

EXPECTED VALUES

The expected ranges for the ARCHITECT Estradiol assay were obtained by testing specimens drawn from 101 males, 72 postmenopausal females and normal menstruating females. For the normal menstruating female ranges, specimens were obtained from 36 women drawn throughout their cycle, resulting in a total of 956 specimens. Variations in cycle length were normalized by aligning the cycles based on Day 0 as the day of the LH peak (same day as the FSH peak and same day or one day after the estradiol peak). To establish cycle-specific reference ranges, the specimens were categorized as follicular phase, mid-cycle phase and luteal phase. Follicular phase was defined as the period of time from 15 days to 2 days prior (-15 to -2) to the period of the mid-cycle gonadotropin surge (Days -1 to +1). The luteal phase was defined as +2 days to +15 days.¹⁷ All cycles included in establishing reference ranges were ovulatory.

The results are presented below.*

Population	n	Estradiol Concentration Value	
		Median (pg/mL)	Central 95% Range (pg/mL)
Normal Menstruating Females			
Follicular Phase	385	54	21 - 251
Mid-Cycle Phase	105	196	38 - 649
Luteal Phase	466	99	21 - 312
Postmenopausal Females not on HRT	50	<10	<10 - 28
Postmenopausal Females on HRT**	22	28	<10 - 144
Males	101	23	11 - 44

* Representative data; results in individual laboratories may vary from these data.

** For n=22, the central 95% range is the same as the range from minimum to maximum.

It is recommended that each laboratory establish its own expected ranges which may be unique to the population it serves depending on the geographical, patient, dietary or environmental factors.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

The ARCHITECT Estradiol assay precision is ≤ 5 pg/mL (total SD) for concentrations in the range of the low control (target 45 pg/mL), and $\leq 7\%$ (total CV) for concentrations in the range of the medium control (target 190 pg/mL) and the high control (target 600 pg/mL).

A study was performed for the ARCHITECT Estradiol assay based on guidance from **CLSI** protocol EP5-A.¹⁸ Three control levels (low, medium, and high) were assayed, using three lots of reagents, in replicates of two at two separate times per day for 20 days on two instruments. Data from this study are summarized below.*

Control	Reagent Lot	Instrument	n	Mean Conc. Value (pg/mL)	Within Run		Total	
					SD	%CV	SD	%CV
Low	1	1	80	48	2.6	5.5	3.3	6.7
Low	1	2	80	47	2.4	5.0	2.8	6.0
Low	2	1	80	45	2.9	6.4	3.3	7.4
Low	2	2	80	46	2.1	4.6	2.6	5.6
Low	3	1	80	45	2.7	6.0	3.0	6.6
Low	3	2	80	47	2.3	4.8	3.3	7.1
Medium	1	1	80	188	3.5	1.9	4.0	2.1
Medium	1	2	80	188	3.6	1.9	4.3	2.3
Medium	2	1	80	188	4.2	2.3	4.6	2.4
Medium	2	2	80	190	3.3	1.7	4.3	2.3
Medium	3	1	80	183	3.7	2.0	4.5	2.5
Medium	3	2	80	192	3.0	1.5	4.3	2.3
High	1	1	80	596	10.5	1.8	10.5	1.8
High	1	2	80	593	8.5	1.4	10.6	1.8
High	2	1	80	606	9.8	1.6	11.5	1.9
High	2	2	80	604	10.3	1.7	10.9	1.8
High	3	1	80	590	10.9	1.8	13.7	2.3
High	3	2	80	591	10.7	1.8	15.2	2.6

* Representative data; results in individual laboratories may vary from these data.

Analytical Sensitivity

The analytical sensitivity of the ARCHITECT Estradiol assay is ≤ 10 pg/mL. Analytical sensitivity is defined as the concentration at two standard deviations from the mean RLU value of the ARCHITECT Estradiol Calibrator A (0 pg/mL) and represents the lowest measurable concentration of estradiol that can be distinguished from zero.

Functional Sensitivity

The functional sensitivity of the ARCHITECT Estradiol assay is ≤ 25 pg/mL.

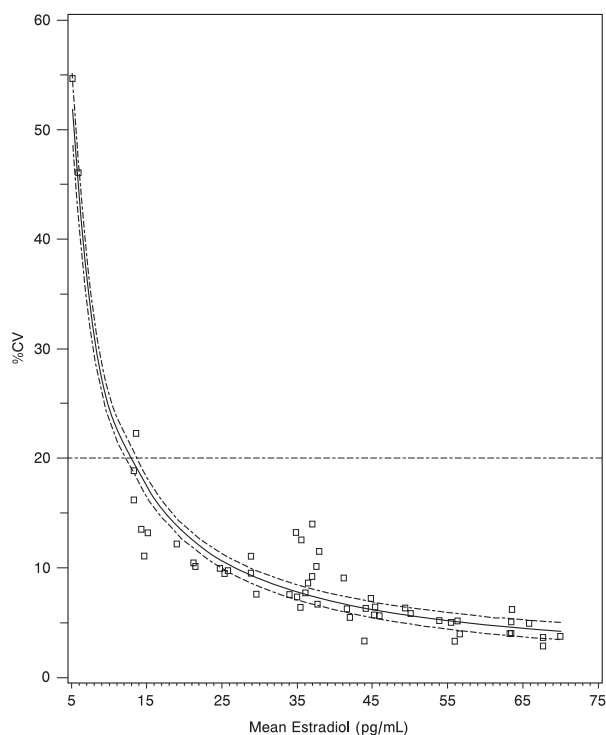
Functional sensitivity is defined as the lowest concentration that can be measured with a coefficient of variation (CV) less than or equal to 20%.

A study was conducted on two instruments and three reagent lots using human panels which were prepared at target concentrations ranging from 0 to 70 pg/mL. The panels were assayed in replicates of three over five days for a total of 15 replicates per panel, instrument and lot combination. Mean and %CV were calculated for each of the 15 replicate combinations. For each instrument, the calculated %CVs were plotted against the corresponding means for all panels and all three reagent lots. A reciprocal curve ($Y=a+b/X$) was fitted through the data and the functional sensitivity was estimated as the concentration corresponding to the 20% CV level of the fitted curve.

The following functional sensitivities were determined:*

Instrument	Functional Sensitivity
A	13 pg/mL
B	14 pg/mL

The precision profile corresponding to the functional sensitivity determination for instrument A is shown below.



--- 20%CV ——— Fitted Curve - - - 95% Confidence Limits of Fitted Curve

* Representative data; results in individual laboratories may vary from these data.

Specificity

The specificity of the ARCHITECT Estradiol assay was determined by studying the compounds listed below in either the absence or presence of estradiol using guidance from **CLSI** protocol EP7-A.¹⁹

Table A*

A study was performed in which synthetic specimens containing essentially no residual estradiol were supplemented with potential cross reactants at the concentrations listed and tested for estradiol. The percent cross reactivity is shown below:

Cross Reactant	Concentration Cross Reactant	% Cross Reactivity
17 β -Estradiol 3-sulfate	50 ng/mL	0.1%
Estrone	1500 pg/mL	0.7%

Cross Reactivity of the following compounds was undetectable at the concentrations listed below:

Cross Reactant	Concentration Cross Reactant
Aldosterone	10 μ g/mL
5 α -Androstan-3 β ,17 β -diol	10 ng/mL
5 α -Androstandione	10 ng/mL
Androstenedione	100 ng/mL
Clomiphene citrate	60 ng/mL
Corticosterone	570 ng/mL
Cortisone	500 ng/mL
Deoxycorticosterone acetate	500 ng/mL
11-Deoxycortisol	500 ng/mL

Cross Reactant	Concentration	Cross Reactant
Dexamethasone	12,770	ng/mL
DHEA	120	ng/mL
DHEAS	8	µg/mL
5β-Dihydrocorticosterone	500	ng/mL
DHT (Dihydrotestosterone)	2	ng/mL
Equilin	0.6	ng/mL
Equilin Sulfate	5	ng/mL
Estetrol	2.4	ng/mL
17α Estradiol	0.3	ng/mL
17β-Estradiol-3-glucuronide	4.8	ng/mL
17β-Estradiol 17-valerate	1	ng/mL
17β-Estradiol 17-propionate	1	ng/mL
17β-Estradiol 3-sulfate/17-glucuronide	50	ng/mL
Estriol	2500	pg/mL
Estriol 16α-(β-D-glucuronide)	106	ng/mL
Estriol 3-sulfate	300	ng/mL
Estriol 3-(β-D-glucuronide)	106	ng/mL
Estrone 3-sulfate	0.4	ng/mL
Ethinodiol diacetate	1	µg/mL
Ethinylestradiol	0.4	ng/mL
Hydrocortisone	500	ng/mL
16α-Hydroxyestrone	1	ng/mL
17α-Hydroxypregnanolone	480	ng/mL
17α-Hydroxyprogesterone	1200	ng/mL
Medroxyprogesterone	12.3	ng/mL
Mestranol	0.4	ng/mL
Norethindrone	16	ng/mL
Norethindrone acetate (Norethisterone acetate)	14	ng/mL
Pregnanolone	59	ng/mL
Progesterone	500	ng/mL
Tamoxifen	183	ng/mL
Testosterone	20	ng/mL

* Representative data; results in individual laboratories may vary from these data.

Table B*

The ARCHITECT Estradiol assay recovery in the presence of the following compounds is 100 ± 40% at the concentrations listed below:

A study was performed in which synthetic specimens containing estradiol (600 pg/mL) were supplemented with potential interferents at the concentrations listed and tested for estradiol. The percent recovery is shown below:

Interferent	Concentration	% Recovery
Equilin	1.2 ng/mL	98.4
Equilin Sulfate	10 ng/mL	92.6
Ethinylestradiol	0.8 ng/mL	88.6
Mestranol	0.8 ng/mL	100.5
Norethindrone	32 ng/mL	76.9
Norethindrone acetate (Norethisterone acetate)	28 ng/mL	99.5

The ARCHITECT Estradiol assay recovery in the presence of the following compounds is 100 ± 10% at the concentrations listed below:

A study was performed in which synthetic specimens containing estradiol (600 pg/mL) were supplemented with potential interferents at the concentrations listed and tested for estradiol. The percent recovery is shown below:

Interferent	Concentration	% Recovery
Aldosterone	10 µg/mL	100.1
5α-Androstan-3β,17β-diol	10 ng/mL	98.6
5α-Androstandione	10 ng/mL	99.6
Androstenedione	100 ng/mL	100.1
Clomiphene citrate	60 ng/mL	98.8
Corticosterone	570 ng/mL	99.1
Cortisone	500 ng/mL	98.4
Deoxycorticosterone acetate	500 ng/mL	98.9
11-Deoxycortisol	500 ng/mL	100.4
Dexamethasone	12,770 ng/mL	100.5
DHEA	120 ng/mL	99.8
DHEAS	8 µg/mL	100.2
5β-Dihydrocorticosterone	500 ng/mL	100.9
DHT (Dihydrotestosterone)	2 ng/mL	100.9
Estetrol	2.0 ng/mL	93.0
17α Estradiol	0.3 ng/mL	100.7
17β-Estradiol-3-glucuronide	4.8 ng/mL	98.8
17β-Estradiol 17-valerate	1 ng/mL	100.4
17β-Estradiol 17-propionate	1 ng/mL	100.1
17β-Estradiol 3-sulfate	50 ng/mL	105.1
17β-Estradiol 3-sulfate/17-glucuronide	50 ng/mL	99.6
Estriol 16α-(β-D-glucuronide)	106 ng/mL	101.3
Estriol 3-sulfate	300 ng/mL	97.9
Estriol 3-(β-D-glucuronide)	106 ng/mL	100.1
Estrone 3-sulfate	0.4 ng/mL	100.1
Ethinodiol diacetate	1 µg/mL	97.7
Hydrocortisone	500 ng/mL	99.4
16α-Hydroxyestrone	1 ng/mL	100.2
17α-Hydroxypregnanolone	480 ng/mL	100.0
17α-Hydroxyprogesterone	1200 ng/mL	98.9
Medroxyprogesterone	12.3 ng/mL	99.1
Pregnanolone	59 ng/mL	100.2
Progesterone	500 ng/mL	100.5
Tamoxifen	183 ng/mL	100.9
Testosterone	20 ng/mL	98.1

* Representative data; results in individual laboratories may vary from these data.

Table C*

The ARCHITECT Estradiol assay recovery in the presence of the following compounds is 100 ± 10% at the concentrations listed below:

A study was performed in which synthetic specimens containing estradiol (concentrations listed below) were supplemented with potential interferents at the concentrations listed and tested for estradiol. The percent recovery is shown below:

Interferent	Concentration Estradiol	Concentration Interferent	% Recovery
Estrone	750 pg/mL	300 pg/mL	93.9
Estrone	4000 pg/mL	1500 pg/mL	92.8
Estriol	4000 pg/mL	1500 pg/mL	98.4
Estriol	150 pg/mL	2500 pg/mL	92.1

* Representative data; results in individual laboratories may vary from these data.

Interference

Potential interference in the ARCHITECT Estradiol assay from hemoglobin, bilirubin, triglycerides, protein, and cholesterol at the levels indicated below is $\leq 10\%$. Interference was evaluated in a study based on guidance from CLSI protocol EP7-A.¹⁹

- Hemoglobin at 500 mg/dL
- Bilirubin at 20 mg/dL
- Triglycerides at 1000 mg/dL
- Protein at 4 and 12 g/dL
- Cholesterol at 240 mg/dL

Method Comparison

The slope of the ARCHITECT Estradiol assay versus isotope dilution-gas chromatography/mass spectrometry (ID-GCMS)²⁰ is 1.00 ± 0.10 from 10 to 1000 pg/mL and 1.00 ± 0.15 from 10 to 5,000 pg/mL. The correlation coefficient is ≥ 0.95 for both ranges.

A study was performed based on guidance from CLSI EP9-A2.²¹ The ARCHITECT Estradiol assay was compared to isotope dilution-gas chromatography/mass spectrometry (ID-GCMS). Specimens with known GCMS concentrations were assayed using the ARCHITECT Estradiol assay. Due to the limited number of GCMS specimens, each specimen was tested with three different reagent lots; one replicate per lot. The mean of the three replicates for each specimen was calculated. The calculated mean was used for the pooled regression analysis. In addition, the correlation coefficient from individual replicates vs. GCMS and the regression statistics from each reagent lot were also calculated. The regression analysis was performed by both Least Squares and Passing-Bablok²². Data from this study are summarized below.*

**Abbott ARCHITECT Estradiol vs. ID-GCMS
(Individual Specimens)**

Range	n	Min GCMS Conc.	Max GCMS Conc.	Correlation Coefficient
10 to 1000 pg/mL	315	13.3	945.2	0.99
10 to 5000 pg/mL	393	13.3	4269.2	0.99

Regression Method	n	Pooled Intercept	Pooled Slope	Slope per Lot (Lot 1, Lot 2, Lot 3)
10 to 1000 pg/mL				
Least Squares	105	0	1.03	0.99, 1.03, 1.08
Passing-Bablok**	105	2	1.02	0.98, 1.02, 1.06
10 to 5000 pg/mL				
Least Squares	131	-31	1.09	1.08, 1.08, 1.12
Passing-Bablok**	131	0	1.04	1.00, 1.03, 1.07

* Representative data; results in individual laboratories may vary from these data.

** A linear regression method with no special assumptions regarding the distribution of the samples and the measurement errors.

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