Access
Immunoassay Systems

ESTRADIOL

REF 33540 **FOR USE WITH TEST NAME: E2**

Intended Use

The Access Estradiol assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of estradiol levels in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

Estradiol (17β - estradiol, 1,3,5 (10) - Estratrien - 3,17β - diol) is a natural estrogen with a molecular mass of 272.3 daltons. Most circulating estradiol is bound to protein. It is estimated that only 1–3% of estradiol is free (unbound). In non-pregnant women, estradiol is secreted by the ovary and the corpus luteum. The adrenals and testes (in men) are also believed to secrete minute amounts of estradiol.[1] Estradiol levels are lowest at menses and into the early follicular phase and rise in the late follicular phase to a peak just prior to the hLH (human Luteinizing Hormone) surge, initiating ovulation. As the hLH peaks, the levels of estradiol decrease before rising again in the luteal phase. Endometrial growth is stimulated by estradiol and progesterone (secreted by the corpus luteum) in preparation for implantation of a fertilized egg. If conception does not occur, the secretion of estradiol and progesterone by the corpus luteum decreases, initiating menses.[2]

Levels of estradiol are used to monitor ovulatory status. Because estradiol levels reflect follicular maturation, the measurement of estradiol as cited in the scientific literature has been used as a valuable tool in the assessment of sexual development, etiology of amenorrhea, causes of infertility and menopause.[3,4] Abnormally high levels in males are indicative of feminizing syndromes such as gynecomastia.[5]

Principles of the Procedure

The Access Estradiol assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with paramagnetic particles coated with goat anti-rabbit: rabbit anti-estradiol and a TRIS-buffered protein solution. After 20 minutes, estradiol alkaline phosphatase conjugate is added. Estradiol in the sample competes with the estradiol-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-estradiol antibody. Resulting antigen: antibody complexes are bound to the capture antibody on the solid-phase. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of estradiol in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

Product Information

Access Estradiol Reagent Pack

Cat. No. 33540: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C for 14 days after initial use.
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.

- If the reagent pack is damaged (i.e., broken elastomer), discard the pack.
- All antisera are polyclonal unless otherwise indicated.

R1a:	Paramagnetic particles coated with goat anti-rabbit IgG: rabbit anti-estradiol in TRIS buffered saline, with bovine serum albumin (BSA), < 0.1% sodium azide.
R1b:	TRIS, sodium chloride, protein (bovine, goat) and < 0.1% sodium azide.
R1c:	Estradiol-alkaline phosphatase conjugate (bovine), protein (BSA, rabbit) < 0.1% sodium azide.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]
- The Material Safety Data Sheet (MSDS) is available upon request.

Specimen Collection and Preparation

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[7]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.

Materials Provided

R1 Access Estradiol Reagent Packs

Materials Required But Not Provided

1. Access Estradiol Calibrators
   Provided at zero and approximately 106, 570, 1800, 3100 and 4800 pg/mL (389, 2092, 6608, 11,380 and 17,621 pmol/L).
   Cat. No. 33545
2. Quality Control (QC) materials: commercial control material.

3. Access Substrate
   Cat. No. 81906
4. **Access, Access 2, SYNCHRON LXi:**
   Access Wash Buffer II, Cat. No. A16792
   UniCel DxI:
   UniCel DxI Wash Buffer II, Cat. No. A16793
5. Access Estradiol Calibrator S0
   Cat. No. 33546

Procedural Comments

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.
3. Use thirty-five (35) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
4. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 3.671.

Procedure

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

Calibration Details

An active calibration curve is required for all tests. For the Access Estradiol assay, calibration is required every 14 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Quality Control

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[8] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

Results

Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

Limitations of the Procedure

1. Samples can be accurately measured within the analytical range of the lower limit of detection and the highest calibrator value (approximately 20–4800 pg/mL [73–17,621 pmol/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 20 pg/mL [< 73 pmol/L]).
   - If a sample contains more than the stated value of the highest Access Estradiol Calibrator (S5), report the result as greater than that value (i.e., > 4800 pg/mL [> 17,621 pmol/L]). Alternatively, dilute one volume of sample with one volume of Access Estradiol Calibrator S0 (zero) which is also available as Access Estradiol Calibrator S0, Cat. No. 33546. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.
2. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples.[9,10]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Estradiol results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.
4. Estradiol values from pregnant females may be affected by high levels of estriol such as are present in the second and third trimesters of pregnancy.[11] Control materials or survey samples containing high levels of estriol may be similarly affected.

Expected Values

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Estradiol concentrations were measured in human serum samples from apparently healthy adult male and female subjects using the Access Estradiol assay. The observed ranges of estradiol concentrations are shown below for each population represented:

Reference Group	n	Median (pg/mL)	Lower Limit (95% Confidence Interval) (pg/mL)	Upper Limit (95% Confidence Interval) (pg/mL)
Males	129	23	< 20	47 (43–53)
Post-menopausal females†	122	< 20	< 20	40 (33–48)
Non-pregnant females				
–mid-follicular phase††	96	57	27 (23–31)	122 (107–139)
–mid-luteal phase†††	100	126	49 (42–57)	291 (250–338)
–peri-ovulatory phase††††	92	198	95 (83–109)	433 (378–495)

† not on hormone therapy

†† range represents days -6 to -8 from the hLH peak (day 0)

††† range represents days +6 to +8 from hLH peak (day 0)

†††† range represents day -1 from the hLH peak (day 0)

Specific Performance Characteristics

Methods Comparison

A comparison of 246 serum values using the Access Estradiol assay on the Access Immunoassay system and a commercially available enzyme immunoassay system gave the following statistical data using Deming calculations:

n	Range of Observations (pg/mL)	Intercept (95% Confidence Interval) (pg/mL)	Slope (95% Confidence Interval)	Correlation Coefficient (r)
246	20–4233	-41.49 (-56.33 to -26.65)	1.07 (1.04–1.09)	0.99 (0.99–1.00)

A comparison of 63 serum values using the Access Estradiol assay on the Access Immunoassay system and ID-GC/MS (Isotope Dilution-Gas Chromatography/Mass Spectrometry)[12,13,14,15] gave the following statistical data using Deming calculations:

n	Range of Observations (pg/mL)	Intercept (95% Confidence Interval) (pg/mL)	Slope (95% Confidence Interval)	Correlation Coefficient (r) (95% Confidence Interval)
63	23.4–4542.3	-6.2 (-36.9 to 24.6)	1.02 (0.999–1.044)	1.00 (0.994–0.998)

A comparison of 151 paired serum and plasma samples, both analyzed using the Access Estradiol assay on the Access Immunoassay system gave the following statistical data using Deming Calculations:

n	Range of Observations (pg/mL)	Intercept (95% Confidence Interval) (pg/mL)	Slope (95% Confidence Interval)	Correlation Coefficient (r) (95% Confidence Interval)
151	13–4096	5.2 (-20.5 to 31.0)	0.99 (0.97–1.01)	0.99 (0.989–0.994)

Dilution Recovery (Linearity)

Dilution of 10 human serum samples containing elevated estradiol levels with Access Estradiol Calibrator S0 (zero) at a 1:2 dilution resulted in a mean recovery of 110%.

Sample ID	Neat Sample (pg/mL)	Expected Concentration (pg/mL)	Actual Concentration (pg/mL)	Recovery (%)
1	707.46	353.73	389.21	110
2	1058.63	529.31	572.13	108
3	1219.55	609.77	703.52	115
4	3306.24	1653.12	1788.35	108
5	1951.20	975.60	1082.36	111
6	2623.87	1311.94	1419.23	108
7	715.26	357.63	398.03	111
8	3082.34	1541.17	1611.38	105
9	2078.44	1039.22	1175.35	113
10	2185.06	1092.53	1230.77	113
			Mean % Recovery	**110**

Patient Sample

Further dilution of elevated estradiol human serum samples with Access Estradiol Calibrator S0 (zero) gave the following results:

Sample #11	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	980.92	980.92	-
1/2	490.46	586.63	120
1/3	327.04	403.5	123
1/4	245.23	295.51	121
1/8	122.61	143.59	117
		Mean % Recovery	**120**

Sample #12	Expected Concentration (pg/mL)	Determined Concentration (pg/mL)	Recovery (%)
Neat	2376.88	2376.88	-
1/2	1188.44	1230.77	104
1/3	792.45	875.52	110
1/4	594.22	656.95	111
1/8	297.11	348.79	117
		Mean % Recovery	**110**

Spiking Recovery

Known amounts of estradiol were added to four human serum samples. The concentration of estradiol was determined before and after the addition of exogenous estradiol and the percent recovery was calculated.

Sample ID	Endogenous level of estradiol (pg/mL)	Expected estradiol level (endogenous plus spike) (pg/mL)	Observed estadiol level (pg/mL)	Recovery (%)
1-A	26	126	134	106
1-B	26	176	195	111
1-C	26	626	661	106
1-D	26	1226	1455	119
2-A	37	137	128	93
2-B	37	187	169	91
2-C	37	637	583	92
2-D	37	1236	1089	88
3-A	67	166	151	91
3-B	67	216	216	100
3-C	67	666	667	100
3-D	67	1266	1340	106
4-A	89	188	211	113
4-B	89	237	274	115
4-C	89	688	827	120
4-D	89	1287	1420	110
			Mean % Recovery	**104**

Imprecision

A polynomial regression of all imprecision data (8 samples spanning the range of the assay), generated from 3 reagent lots and analyzed on multiple instruments, provides an overall estimation of the imprecision. Data was analyzed by plotting the log of the sample

concentration by the log of the standard deviation and determining the upper limit of the 95% confidence interval of this regression fit. This assay exhibits total imprecision of ≤ 12% at concentrations ≥ 120 pg/mL. The following table was estimated from the polynomial regression.

Concentration (pg/mL)	CV	Estimated SD
50	21%	10.5
60	19%	11.4
80	15%	12.0
100	13%	13.0
120	12%	14.4
>120	≤ 12%	--

Analytical Specificity/Interferences

Serum samples containing up to 10 mg/dL (171 µmol/L) Bilirubin, hemolyzed samples up to 1 g/dL (10 g/L) hemoglobin and lipemic samples containing the equivalent of 1800 mg/dL (20.32 mmol/L) triglycerides do not affect the concentration of estradiol assayed utilizing a serum sample containing approximately 1089 pg/mL estradiol.

The following potential cross-reactants of substances that are similar in structure to estradiol did not exceed 5% cross-reactivity:

- Estrone sulfate
- Estrone
- Estriol
- Estriol 3-sulfate
- Estriol 17-sulfate
- Ethinyl estradiol
- Estradiol valerate
- Aldosterone
- Testosterone
- Androstenediol
- 17-α-estradiol
- 17β-Estradiol 3 glucuronide
- 3,17β-Estradiol diglucuronide
- Norgestrel
- Estrone-3-glucuronide

Data observed for analytical specificity/interferences testing are provided below:

Substance	Amount Added (pg/mL)	Amount Observed (pg/mL)	Apparent Cross-Reactivity (%)
Estrone sulfate	1,000,000	63.35	0.01
Estrone	20,000	395.28	1.98
Estriol	10,000	49.69	0.50
Estriol 3-sulfate	2,500,000	0.00	ND†
Estriol 17-sulfate	2,500,000	59.61	0.002
Ethinyl estradiol	50,000	184.76	0.37
Estradiol valerate	1,000,000	2850.97	0.29
Aldosterone	100,000,000	0.00	ND†
Testosterone	10,000,000	446.89	0.004
Androstenediol	2,000,000	22.53	0.001
17-α-estradiol	100,000	353.81	0.35
17β-Estradiol 3 glucuronide	2,000,000	574.19	0.029
3,17β-Estradiol diglucuronide	2,000,000	9.97	ND†
Norgestrel	100,000,000	506.14	0.00051
Estrone-3-glucuronide	100,000,000	634.80	0.0006

†ND: no significant statistical difference was observed.

Analytical Sensitivity

The lowest detectable level of estradiol distinguishable from zero (Access Estradiol Calibrator S0) with 95% confidence is 20 pg/mL (73 pmol/L). This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the fitted zero calibrator signal.

Access
Immunoassay Systems

ESTRADIOL CALIBRATORS

REF 33545

Intended Use

The Access Estradiol Calibrators are intended to calibrate the Access Estradiol assay for the quantitative determination of estradiol levels in human serum and plasma using the Access Immunoassay Systems.

Summary and Explanation

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability

The measurand (analyte) in the Access Estradiol Calibrators is traceable to ID/GC-MS (Isotope Dilution-Gas Chromatography/Mass Spectrometry).[12,13,14,15] Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Product Information

Access Estradiol Calibrators

Cat. No. 33545: S0, 4.0 mL/vial; S1–S5, 2.5 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Signs of possible deterioration are control values out of range.
- Refer to calibration card for exact concentrations.

S0:	Human serum, < 0.1% sodium azide, and 0.025% Cosmocil** CQ. Contains 0 pg/mL (pmol/L) estradiol.
S1, S2, S3, S4, S5:	Estradiol (purified chemical compound) in human serum at levels of approximately 106, 570, 1800, 3100 and 4800 pg/mL (389, 2092, 6608, 11,380 and 17,621 pmol/L), respectively, with < 0.1% sodium azide, and 0.025% Cosmocil CQ.
Calibration Card:	1

Warnings and Precautions

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[16]

- Each serum/plasma pool used in the preparation of this product has been tested and found negative for the presence of fibrinogen.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]
- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibration Details

The Access Estradiol Calibrators are provided at six levels – zero and approximately 106, 570, 1800, 3100 and 4800 pg/mL – prepared from synthetic estradiol and human serum. Assay calibration data are valid up to 14 days.

Run the Access Estradiol S0 and S1 Calibrators in quadruplicate, and the S2–S5 Calibrators in duplicate.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

Access
Immunoassay Systems

ESTRADIOL CALIBRATOR S0

REF 33546

Intended Use

The Access Estradiol Calibrator S0 is intended for use with Access Estradiol assay to dilute patient samples containing analyte concentrations greater than the analyte specific S5 calibrator.

Summary and Explanation

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

Product Information

Access Estradiol Calibrator S0

Cat. No. 33546: 4 mL/vial

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.

S0:	Human serum, < 0.1% sodium azide, and 0.025% Cosmocil** CQ. Contains 0 pg/mL (pmol/L) estradiol.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[16]
- Each serum/plasma pool used in the preparation of this product has been tested and found negative for the presence of fibrinogen.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[6]
- The Material Safety Data Sheet (MSDS) is available upon request.

Procedure

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

Limitations of the Procedure

If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

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