17-α-OH Progesterone ELISA

For the quantitative measurement of 17-α-OH Progesterone (17-α-OHP) in serum or plasma (EDTA, heparin, or citrate)

For "*In Vitro* Diagnostic" use within the United States of America. This product is for "Research Use Only" outside of the United States of America.

Catalog Number: 20-17OHU-E01
Size: 96 Wells
Version: 18.0, 2023-10-05 - ALPCO 3.0

1. Introduction

Intended Use

The **17-α-OH Progesterone ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of 17-α-OH Progesterone (17-α-OHP) in serum or plasma (K_2-EDTA, K_3-EDTA, lithium heparin, or citrate plasma 3.2%). **For in vitro diagnostic use within the United States.** This product is for Research Use Only outside of the United States of America.

1.1. Summary and Explanation

The steroid 17-α-hydroxyprogesterone (17-α-OHP) is produced by both the adrenal cortex and gonads. Even though 17-α-OHP has relatively little progestational activity, it is of intense clinical interest because it is the immediate precursor to 11-deoxycortisol (Cpd-S). Because Cpd-S is produced by 21-hydroxylation of 17-α-OHP, measurement of 17-α-OHP is a useful indirect indicator of 21-hydroxylase activity. In congenital 21-hydroxylase deficiency, the most common variety of congenital adrenal hyperplasia (CAH), 17-α-OHP is secreted in abundant excess. It is moderately elevated in 11-β-hydroxylase deficiency as well. Measurement of 17-α-OHP is therefore valuable in the initial diagnosis of CAH.

1.2. Clinical Physiology

Adult non-pregnant women: In adult non-pregnant women in the childbearing age group, 17-α-OHP concentrations vary over the menstrual cycle with luteal phase concentrations being higher than follicular phase concentrations. This is because 17-α-OHP is secreted in parallel with progesterone from maturing follicles or from the corpus luteum. There is also a diurnal variation of 17-α-OHP concentrations. This rhythm is in parallel with adrenal cortisol secretion such that maximum 17-α-OHP concentrations are measured in samples obtained between midnight and 8:00 am.

Adult males: Little information is available on the systematic variability of 17-α-OHP concentration in adult males.

Pregnant women and newborn children: The steroid 17-α-OHP is produced in large amounts by the fetus and the adrenals. It is secreted in abundance into both the fetal and maternal circulation. The maternal concentrations of 17-α-OHP increase very sharply after 32 weeks gestational age to about 4-fold above basal concentrations at term.

1.3. Clinical Applications

Congenital adrenal hyperplasia: The principal application of the 17-α-OHP EIA is in the diagnosis of CAH in newborns with ambiguous genitalia and in virilized adolescent girls. Since 17-α-OHP is the immediate precursor to 11-deoxycortisol, basal 17-α-OHP concentrations are sharply elevated in patients with 21-hydroxylase deficiency and to a lesser degree in patients with 11-hydroxylase deficiency. Because 17-α-OHP concentrations are markedly elevated in newborns and adolescent girls afflicted with CAH, a single basal measurement is all that is normally required to make the diagnosis.

The reported screening incidence for classical CAH in Europe generally varies from 1:10,000 to 1:14,000, similar to the reported incidence results in North America of 1:15,000 to 1:16,000. Higher incidences were noted in Brazil (1:7,500), La Reunion (1:4,000), and Alaska (1:288 in Yupik Eskimos, and 1:800 in Native Alaskans). (13,14). A very high concentration of 17-hydroxyprogesterone at 3 days in full-term infant is diagnostic of classic 21-hydroxylase deficiency (15). Typically, salt-losing patients have higher 17-hydroxyprogesterone

concentrations than non-salt-losing patients. False-positive results from neonatal screening are common with premature infants, and many screening programs have established reference ranges that are based on weight and gestational age. A corticotropin stimulation test (ACTH) can be used to assess borderline cases. Genetic analysis can be helpful to confirm the diagnosis. Randomly measured 17-hydroxyprogesterone concentrations can be normal in patients with nonclassical CAH. Thus, the gold standard for diagnosis of the non-classic form is a corticotropin stimulation test, with measurement of 17-hydroxyprogesterone at 60 min.

Late onset adrenal hyperplasia: More recently, 17-α-OHP concentrations have been utilized in the evaluation of androgenized women where late onset of 21-hydroxylase deficiency is suspected. This condition is clinically very subtle and since the presentation is the same as classical polycystic ovarian disease, basal plasma 17-α-OHP concentrations, unlike classical congenital adrenal hyperplasia, are normal. The diagnosis is made by administration of an ACTH stimulation test.

Other applications: Measurement of 17-α-OHP concentrations is also utilized in evaluation of both men and women with acne vulgaris, male pattern baldness and in some forms of infertility. Experiences with these applications are very limited.

2. Principle of The Test

The 17-α-OH Progesterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with a polyclonal [rabbit] antibody directed towards an antigenic site of the 17-α-OHP molecule. Samples are pre-incubated in the coated wells. During the second incubation, 17-α-OHP in samples compete with a 17-α-OHP-horseradish peroxidase conjugate for binding to the coated antibody. After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of color is inversely proportional to the concentration of 17-α-OHP in the sample. A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using the standard curve.

3. Warnings and Precautions

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Do not reuse microtiter wells.
4. Reagents from other manufacturers must not be used together with reagents of this test kit.
5. All reagents in this kit are clear liquids, substrate solution is clear and colorless. Changes in its appearance may affect the performance of the test. In this case, contact ALPCO.
6. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
7. The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
8. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
9. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn the solution colored. Do not pour reagents back into vials as reagent contamination may occur.
10. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
11. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

12. Allow the reagents to reach room temperature (20-26°C) before starting the test. Temperature will affect the absorbance readings of the assay.
13. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
14. Do not smoke, eat, drink, or apply cosmetics in areas where samples or kit reagents are handled.
15. Wear lab coats and disposable gloves when handling samples and reagents and where necessary safety glasses.
16. Microbial contamination of reagents or samples may give false results.
17. Handling should be done in accordance with the procedures defined by appropriate national biohazard safety guidelines or regulations.
18. Do not use reagents beyond expiry date as shown on the kit labels.
19. All indicated volumes must be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
20. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may cause slightly different results.
21. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
22. Some reagents contain preservatives in non-declarable concentrations. Nevertheless, in case of contact with eyes or skin, flush immediately with water.
23. Substrate Solution contains an ingredient in non-declarable concentrations which causes serious eye irritation. In case of possible contact with eyes, rinse immediately, carefully, and thoroughly with eye wash or water. After contact with skin, wash with plenty of water. Take-off contaminated clothing and wash it before reuse.
24. Chemicals and prepared or used reagents must be treated as hazardous waste according to national biohazard safety guidelines or regulations.
25. The device contains material of animal origin, which is certified apparently free of infectious or contagious diseases and injurious parasites.
26. Bovine components originate from countries where BSE (Bovine spongiform encephalopathy) has not been reported.
27. All materials and samples of human or animal origin must be handled as if capable of transmitting infectious diseases.
28. Handling must be done in accordance with the procedures defined by appropriate national and local biohazard and safety guidelines or regulations. Waste must be discarded according to local rules and regulations.
29. This product does not contain substances which have carcinogenic, mutagenic, or toxic for reproduction (CMR) properties.
30. The following kit components are classified as hazardous: Standard 0 – 6, Control low, Control high, Enzyme Conjugate, Wash Solution.

 **Warning**	**Hazard statement(s):** H317 - May cause an allergic skin reaction. EUH071 - Corrosive to the respiratory tract
	Precautionary statement(s): P261 - Avoid breathing dust/fume/gas/mist/vapours/ spray. P280 - Wear protective gloves. P333 + P313 - If skin irritation or rash occurs: Get medical advice/attention. P362 + P364 - Take off contaminated clothing and wash it before reuse. P501 - Dispose of contents/ container to an approved waste disposal plant

For information on hazardous substances included in the kit please refer to the Safety Data Sheet.

4. Materials Provided

4.1. Reagents provided

Microtiterwells	12 x 8 wells (break apart)	**Microtiter plate** Coated with anti-17-α-OHP antibody (polyclonal).	Ready to use
Standard (Standard 0-6)	7 vials x 1 mL	**Standards *** Concentrations: 0; 0.15; 0.5; 1.5; 3; 7.5; 20 ng/mL 0; 0.45; 1.5; 4.5; 9.1; 22.7; 60.6 nmol/L Conversion: ng/mL × 3.03 = nmol/L. Calibrated against the following reference material: *Certified Reference Material Cerilliant H-085*	Ready to use
Control Low & Control High	2 x 1 mL	**Controls *** *For control values and ranges please refer to vial label or Certificate of Analysis.*	Ready to use
Enzyme Conjugate	1 x 25 mL	**Enzyme Conjugate *** 17-α-OHP conjugated to horseradish peroxidase; Colored red.	Ready to use
Substrate Solution	1 x 25 mL	**Substrate Solution** Contains 3,3‘,5,5‘-tetramethylbenzidine (TMB). *Keep away from direct sunlight.*	Ready to use
Stop Solution	1 x 14 mL	**Stop Solution** Contains < 5 % H_2SO_4. *Avoid contact with the stop solution. It may cause skin irritations and burns.*	Ready to use
Wash Solution	1 x 30 mL	**Wash Solution, 40X concentrate ***	*See “Reagent Preparation“.*
1 x		**Instructions for Use**	
1 x		**Certificate of Analysis (CoA)**	

* Contain(s) < 0.0015% CMIT/ MIT (3:1)
Abbreviations:
CMIT: 5-chloro-2-methyl-4-isothiazolin-3-one
MIT: 2-methylisothiazol-3(2H)-one

4.2. Materials required but not provided

- A calibrated microtiter plate reader (450 nm with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes (1 µL -1000 µL)
- Manual or automatic equipment for rinsing microtiter plate wells
- Absorbent paper
- Distilled water
- Timer
- Semi-logarithmic graph paper or software for data reduction

4.3. Storage and Stability

- **Unopened kits and reagents** as well as **opened reagents** must be stored at 2 °C to 8 °C.
- The microplate must always be stored in the resealable aluminum pouch containing a desiccant. Do not open the pouch until it has reached room temperature. The microtiter plate consists of 12 individual strips. Each strip can be divided into 8 individual wells.
- Unused wells must be immediately returned to the aluminum pouch with the desiccant and stored again tightly resealed at 2 °C to 8 °C.
- Once opened, reagent vials must be closed tightly again.

	Storage Temperature	Stability
Unopened kits and unopened reagents	2 °C to 8 °C	Until the expiration date printed on the label. Do not use reagents beyond this date!
Opened kit	2 °C to 8 °C	8 weeks

4.4. Reagent Preparation

Bring all reagents and required number of strips to room temperature (20-26°C) prior to use.

Wash Solution

Add distilled water to the 40X concentrated Wash Solution. To prepare the contents of the entire wash bottle: dilute 30 mL of concentrated *Wash Solution* with 1170 mL distilled water to a final volume of 1200 mL. *The 1x working Wash Solution is stable for 1 week at room temperature.*

4.5. Disposal of the Kit

The disposal of the kit and all used materials/reagents must be made according to national and local regulations. Special information for this product is given in the Safety Data Sheet.

4.6. Damaged Test Kits

In case of any severe damage to the test kit or components, ALPCO must be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They must be stored until a final resolution has been found. After this, they should be disposed of according to the official regulations.

5. Sample Collection and Preparation

Serum or plasma (EDTA, lithium heparin, or citrate plasma 3.2%) can be used in this assay. Do not use hemolyzed, icteric, or lipemic samples.
Please note: Samples containing sodium azide should not be used in the assay. For further information, refer to the Interfering Substances Section.

5.1. Sample Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate the serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anticoagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Note: Whole blood should not be frozen before centrifugation.

Stability of whole blood at 20 °C to 26 °C up to 7 days (10,11)

5.2. Sample Storage and Preparation

Samples should be capped tightly and may be stored for up to 7 days at 2 °C to 8 °C prior to assaying. Samples stored for a longer time (up to 12 months) should be frozen in aliquots only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

6. Assay Procedure

6.1. General Remarks

- All reagents and samples must be allowed to come to room temperature (20 °C to 26 °C) before use. All reagents must be mixed without foaming.

- Do not interchange caps of reagent vials to avoid cross-contamination.
- Once the test has been started, all steps should be completed without interruption and in the same sequence for each step.
- Use new disposable plastic pipette tips for each standard, control, or sample to avoid cross contamination.
- Optical density is a function of the incubation time and temperature. Respect the incubations times and temperatures as given in the "Test Procedure" section.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- Do not let wells dry during the assay; add reagents immediately after completing the rinsing steps.
- Mix the contents of the microtiter plate wells thoroughly to ensure good test results.
- **Important note to wash procedure:** Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
- **Test performance using fully automated analysis devices:** Automated test performance using fully automated, open-system analysis devices is possible. However, the combination must be validated by the user.
- Generally, the enzymatic reaction is linearly proportional to time and temperature.

6.2. Test Procedure

Each run must include a standard curve. The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run. The given test procedure describes manual processing.

Important note: The accuracy of this assay is markedly influenced by the correct incubation temperature.

1. Secure the desired number of microtiter wells in the frame holder.
2. Dispense **25 µL** of each **Standard, Control,** and **sample** with new disposable tips into appropriate wells.
3. Incubate for **5 minutes** at room temperature
4. Dispense **200 µL** ***Enzyme Conjugate*** into each well.
5. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
6. Incubate for **60 minutes** at room temperature.
7. Wash the wells as follows: If the wash step is performed manually: Briskly shake out the contents of the wells. Rinse the wells **3 times** with **300 µL** 1X working *Wash Solution* per well. If an automated plate washer is used: Rinse the wells **3 times** with **300 µL** 1X working *Wash Solution* per well. At the end of the washing step, always strike the wells sharply on absorbent paper to remove residual droplets!
8. Add **200 µL** of ***Substrate Solution*** to each well.
9. Incubate for **30 minutes** at room temperature.
10. Stop the enzymatic reaction by adding **100 µL** of ***Stop Solution*** to each well.
11. Measure the optical density (OD) of the solution in each well at **450 nm (measurement wavelength) and at 620 nm or 630 nm (reference wavelength for recommended background subtraction)** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3. Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls, and samples.
2. For duplicate determinations, the mean of the two optical density (OD) values for each standard, control, and sample must be taken. If the two values deviate substantially from one another, it is recommended to retest the sample.
3. Manual method: Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis. Determine the corresponding sample concentration from the standard curve by using the (mean) OD value for each sample.
4. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
5. Automated method: the results in the IFU have been calculated automatically using a 4-Parameter curve fit (4-Parameter Rodbard or 4-Parameter Marquardt are the preferred methods). Other data reduction functions may give slightly different results.
6. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard must be further diluted or reported as > 20 ng/mL. For the calculation of the concentrations this dilution factor must be considered.

6.3.1. Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generation at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	2.15
Standard 1 (0.15 ng/mL)	1.77
Standard 2 (0.5 ng/mL)	1.28
Standard 3 (1.5 ng/mL)	0.77
Standard 4 (3.0 ng/mL)	0.49
Standard 5 (7.5 ng/mL)	0.25
Standard 6 (20 ng/mL)	0.12

7. Expected Normal Values

The values are only for user's guidance.
It is strongly recommended for each laboratory to establish its own specific values that take into consideration a population indigenous to the area where the laboratory is located.
Values above or below the reference range should be considered as suspicious and require additional testing. The results alone must not be the only reason for any therapeutic consequences. The results should be correlated with other clinical observations and diagnostic tests.

In a study conducted with newborns and children using the 17-α-OH Progesterone ELISA, the following values were observed:

Newborns (boys and girls)	n	Month After Birth	Range (min-max) (ng/mL)	Mean (ng/mL)	Median (ng/mL)	2.5th-97.5th Percentile (ng/mL)
	26	1st month after birth	0 - 17.3	7.2	6.7	1.0 - 17.0
	43	2nd month after birth	0.32 - 13.7	4.9	4.6	1.6 - 9.8
	21	3rd month after birth	0.06 - 4.2	2.3	2.3	0.5 - 4.1
	12	4th month after birth	0.2 - 4.6	2.1	2.3	0.2 - 4.3

Population	n	Age (years)	Mean (ng/mL)	Median (ng/mL)	2.5th - 97.5th Percentile (ng/mL)	Range (min - max) (ng/mL)
Children	73	1 - 12	0.95	0.83	0.08 – 1.93	0.03 - 2.13
Middle Adolescent Males	11	13 - 18	1.17	1.23	0.42 – 2.24	0.41 – 2.35
Middle Adolescent Females	-	13 - 18	Refer to the reference values for “Adult females”			

In a study conducted with apparently normal healthy donors using the 17-α-OH Progesterone ELISA, the following values were observed:

Population	N	Mean (ng/mL)	Median (ng/mL)	2.5th – 97.5th Percentile (ng/mL)	Range (min-max) (ng/mL)	Mean (ng/mL)
Adult Females	95	Follicular phase	1.06	1.10	0.44 – 1.60	0.30 – 1.70
	98	Luteal phase	2.28	2.40	0.90 – 4.06	0.70 – 4.60
	39	Ovulation	1.48	1.50	0.49 – 2.32	0.20 – 2.60
	26	Postmenopausal	0.38	0.32	0.17 – 0.83	0.16 – 1.04
Adult Males	50	-	0.99	0.90	0.24 – 2.24	0.16 – 2.54

8. Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the Certificate of Analyses (CoA) present in the kit. The values and ranges stated on the CoA always refer to the current kit lot and must be used for direct comparison of the results.
If available, it is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, patient results should be considered invalid. In this case, please check the following technical areas: pipetting and timing devices, photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above-mentioned items without finding any error contact ALPCO.

9. Performance Characteristics

9.1. Assay Dynamic Range

The range of the assay is between 0.15 – 20 ng/mL.

9.2. Sensitivity

Calculated according to CLSI guideline EP17-A2:2012.

The Limit of Blank (LoB) is 0.047 ng/mL

The Limit of Detection (LoD) is 0.083 ng/mL

The Limit of Quantification (LoQ) is 0.156 ng/mL

9.3 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay. Bias must be < 10%:

Substance	Conc. Range (ng/mL)	Mean Bias %
17-Benzoate Estradiol	2 - 2000	-2.00
17-Cypionate Estradiol	2 - 2000	-4.75
17-Valerate Estradiol	2 - 2000	-5.32
Aldosterone	2 - 2000	-0.15
Androstenedione	2 - 2000	-6.12
Corticosterone	2 - 200	-0.92
Cortisol	2 - 200	-5.68
Cortisone	2 - 200	-1.01
DHEA	2 - 2000	-6.02
DHEA-S	2 - 2000	-1.21
Estradiol	2 - 2000	-4.39
Estriol	2 - 2000	-8.09
Estrone	2 - 2000	-5.94
Progesterone	2 - 20	1.31
Testosterone	2 - 2000	-9.39

No substantial cross-reactivity of the assay to structurally related substances is detected (<±10 %).
A bias > + 10% was found for Progesterone at concentrations ≥ 200 ng/mL.
A bias > + 10% was found for Corticosterone and Cortisol at concentrations ≥ 2000 ng/mL.

9.4. Reproducibility

Designed on the basis of CLSI guideline EP5-A3:2014.

9.4.1. Intra-Assay

The within-assay variability was determined with 4 patient samples covering the complete measuring range in 1 run with 10 replicates. CV was calculated as mean CV of 10 replicates:

Sample	n	Mean (ng/mL)	CV (%)
1 Li-Heparin Plasma	10	0.23	4.6
2 Serum	10	2.10	4.0
3 Serum	10	7.74	3.0
4 Citrate Plasma	10	12.11	4.2

9.4.2. Inter-Assay

The between-assay variability was determined for 4 patient samples covering the measuring range in 3 independent runs on 3 days with 10 determinations. CV was calculated from 30 determinations. CV must be < 15 %:

Sample	n	Mean (ng/mL)	CV (%)
1 Li-Heparin Plasma	30	0.21	8.7
2 Serum	30	1.99	6.3
3 Serum	30	7.41	6.3
4 Citrate Plasma 3.2%	30	11.53	6.2

9.4.3 Inter-Lot

The inter-assay (between lots) variation was determined by 6 measurements of different samples with 3 different kit lots. CV must be < 15 %:

Sample	n	Mean (ng/mL)	CV (%)
1 (K_3 EDTA Plasma)	18	0.30	9.4
2 (K_3 EDTA Plasma)	18	0.86	2.5
3 (Serum)	18	1.88	5.7
4 (serum)	18	7.22	6.7

9.5. Recovery

Recovery was determined by adding increasing amounts of the analyte to different patient samples containing different amounts of endogenous analyte. The percentage recoveries were determined by comparing expected and measured values of the samples.

	Sample 1 Citrate plasma 3.2 %	Sample 2 Li-Heparin plasma	Sample 3 K_3 EDTA plasma	Sample 4 Serum
Highest concentration added (ng/mL)	1.86	1.86	1.86	1.86
Concentration (ng/mL)	0.17	0.46	0.72	1.92
Average Recovery (%)	97.9	96.0	95.7	93.8
Range of Recovery (%) from	92.6	89.2	93.3	90.2
to	103.8	103.2	98.6	96.5

9.6. Linearity

Samples containing different amounts of analyte were serially diluted with *Standard 0*. The percentage recovery was calculated by comparing the expected and measured values for the analyte.

	Sample 1 Citrate plasma 3.2 %	Sample 2 Li-Heparin plasma	Sample 3 K_3 EDTA plasma	Sample 4 Serum
Highest Dilution	1:2	1:128	1:16	1:16
Concentration (ng/mL)	1.31	39.96	10.90	15.20
Average Recovery (%)	104.1	103.1	108.0	105.7
Range of Recovery (%) from	93.9	86.9	100.0	102.4
to	111.4	110.2	113.8	111.1

10. Limitations of Use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

10.1. Interfering Substances

Matrix Interference

No interference (bias < ± 20 %) was found for addition of interferent up to concentration stated in the table below.

Bilirubin unconjugated	0.30 mg/mL
Bilirubin conjugated	0.10 mg/mL
Hemoglobin	4.00 mg/mL
Triglyceride	7.50 mg/mL
Cholesterol	4.00 mg/mL
Ethanol	4.00 mg/mL
Glucose	12.50 mg/mL

10.2. Heterophilic Antibody Interference

The assay format is not sensitive to heterophilic antibodies and it is not affected by poly-specific human anti-mouse antibodies (HAMA) (12). Therefore, there is no need to test these interferences. Nevertheless, complete suppression of their effects cannot be guaranteed (9).

10.3. Autoantibody Interference

Autoantibodies including Rheumatoid Factors (RFs) react like heterophilic antibodies. The assay format is not affected by heterophilic antibodies (12). Therefore, there is no need to test these interferences. Nevertheless, complete suppression of autoantibody effects cannot be guaranteed (9).

10.4. Drug Interferences

The following drugs were tested.
Bias must be < 10 %.

Substance	Concentration	Mean Bias
	ng/mL	ng/mL
Coumestrol	5 – 500	-1.91
Daidzein	0 – 5	8.48
Ethisterone	23.4 – 2340	-9.08
Fulvestrant	5 – 500	2.43
Genistein	5 – 500	4.10
Levonorgestrel	0.2 – 20	6.54
Mifepristone	23.4 – 2340	-9.47
Prednisolone	0.2 – 20	-1.31
Prednisone	0.2 – 2	-9.31
Secoisolariciresinol	5 – 500	8.13

A bias > + 10% was found for Daidzein at concentrations ≥ 50 ng/mL.
A bias > - 10% was found for Prednisone at concentrations ≥ 20 ng/mL.
A bias > - 10% was found for Mifepristone at concentrations ≥ 2340 ng/mL.

10.5. High-Dose-Hook Effect
High Dose Hook Effect is not detected in the range between 0 - 640 ng/mL.

11. Trueness (Bias)
The assay is calibrated in the range of the reference material (Cerilliant; H-085). The difference from the expected value was below ± 20 %.

Concentration ng/mL	Bias	Uncertainty of the reference material
0.78	-4.0%	15.1 %
5.25	-16.0 %	7.8 %
12.5	2.8 %	9.6 %

11.1. Interpretation of Results

11.2. Reliability of Results
The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact ALPCO.

11.3. Therapeutic Consequences
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.2. Any laboratory result is only a part of the total clinical picture of a patient. Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived. The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.4. Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.3 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of ALPCO.

11.4 Reporting of Serious Incident
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority in which the user and/or the patient is established.

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