# Access
Immunoassay Systems

# PROGESTERONE
REF 33550

## Intended Use

The Access Progesterone assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of progesterone levels in human serum using the Access Immunoassay Systems.

## Summary and Explanation

**Physiological occurrence**

Both men and women produce low levels of progesterone, a steroid hormone in the adrenal cortex. Progesterone is important, not only as a hormone, but also as the precursor of the estrogens, androgens, and adrenocortical steroids.[1]

In women, during the follicular phase of the menstrual cycle, serum levels remain low. After ovulation, there is a significant rise in serum levels when the corpus luteum begins to produce progesterone in increasing amounts. This causes changes in the uterus, preparing it for implantation of a fertilized egg. If implantation occurs, the trophoblast begins to secrete hCG (human chorionic gonadotropin), which maintains the corpus luteum and its secretion of progesterone. By the end of the first trimester, the placenta becomes the primary secretor, and serum levels continue to increase. If there is no implantation, the corpus luteum degenerates and circulating progesterone levels decrease rapidly, reaching follicular phase levels about 4 days before the next menstrual period.[2]

**Metabolism and transport**

Steroid syntheses begin with acetate conversion pathways to cholesterol. Pregnenolone is the immediate precursor and 17-α hydroxyprogesterone one of the first metabolites of progesterone.[1,3]

The predominant metabolite in the liver is pregnanediol, generally as a water soluble sulfate or glucuronide, which can then be secreted by the kidneys. Progesterone is transported mainly through binding to albumin, and to a lesser extent corticosteroid binding globulin (CBG) or sex hormone binding globulin (SHBG).[3,4]

**Clinical applications**

In general, increasing progesterone levels are indicative of viable pregnancies. Ultrasonography is required to confirm viability at low progesterone levels. Serum concentrations are relatively constant at 8–10 weeks gestation, unless the pregnancy is failing, which can be signaled by decreasing progesterone values. After 10–12 weeks, levels increase more rapidly, but serum progesterone determinations are not considered useful for diagnoses in late pregnancy.[5,6]

Ovulation, and the presence of a functioning corpus luteum, can be demonstrated with serial determinations of serum progesterone. Luteal phase dysfunction may be diagnosed when ovulation has occurred and there is inadequate luteinization and reduced progesterone secretion.[7,8]

## Principles of the Procedure

The Access Progesterone assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with rabbit antibody to progesterone, progesterone-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-rabbit capture antibody. Progesterone in the sample competes with the progesterone-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-progesterone antibody. Resulting antigen: antibody complexes bind 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 progesterone in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

## Product Information

**Access Progesterone Reagent Pack**

**Cat. No. 33550: 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 28 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:** | Progesterone-alkaline phosphatase (bovine) conjugate and paramagnetic particles coated with goat anti-rabbit IgG in TRIS buffered saline, with bovine serum albumin (BSA), < 0.1% sodium azide, and 0.0125% Cosmocil** CQ. |
| **R1b:** | Protein (goat, rabbit) in acetate buffer with 0.0125% Cosmocil CQ. |
| **R1c:** | Rabbit antiserum to progesterone in acetate buffer, BSA, < 0.1% sodium azide, and 0.0125% Cosmocil CQ. |

## 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.[9]
- The Material Safety Data Sheet (MSDS) is available upon request.

## Specimen Collection and Preparation

1. Serum is the recommended sample. To avoid time related absorption, specimens should not be stored in collection vials with gel separators.[10]
2. Observe the following recommendations for handling, processing, and storing blood samples:[11]
   - 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 has 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.
5. Avoid assaying grossly lipemic samples.

**Materials Provided**

R1 Access Progesterone Reagent Packs

**Materials Required But Not Provided**

1. Access Progesterone Calibrators
   Provided at zero and approximately 1.0, 4.0, 10.0, 20.0, and 40.0 ng/mL (3.18, 12.72, 31.80, 63.60, and 127.20 nmol/L).
   Cat. No. 33555
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 Progesterone Calibrator S0
   Cat. No. 33556

**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 twenty (20) 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 ng/mL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 3.18.

**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**

**Run the Access Progesterone Calibrator S0 in quadruplicate, the S1 calibrator in triplicate**, and the S2–S5 calibrators in duplicate.

An active calibration curve is required for all tests. For the Access Progesterone assay, calibration is required every 28 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.[12] 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 using a weighted four parameter logistic curve (4PLC) math model. 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 analytic range of the lower limit of detection and the highest calibrator value (approximately 0.10–40.0 ng/mL [0.32–127.20 nmol/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., < 0.10 ng/mL [< 0.32 nmol/L]).
   - If a sample contains more than the stated value of the highest Access Progesterone Calibrator (S5), report the result as greater than that value (i.e., > 40.0 ng/mL [> 127.20 nmol/L]). Alternatively, dilute one volume of sample with two volumes of Access Progesterone Calibrator S0 (zero), which is also available as Access Progesterone Calibrator S0 Cat. No. 33556. 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.[13,14]
   Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
3. The Access Progesterone 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.

## Expected Values

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

| Reference Group | n | Mean (ng/mL) | 95% Confidence Interval (ng/mL) |
|---|---|---|---|
| Males | 161 | 0.84 | 0.14–2.06 |

| Reference Group | n | Mean (ng/mL) | Range of Observations (ng/mL) |
|---|---|---|---|
| Non-pregnant females | | | |
| mid- follicular phase | 14 | 0.69 | 0.31–1.52 |
| mid-luteal phase | 13 | 11.42 | 5.16–18.56 |
| post menopausal* | 49 | 0.25 | < 0.08–0.78 |
| Pregnancy | | | |
| first trimester | 34 | 22.17 | 4.73–50.74 |
| second trimester | 29 | 29.73 | 19.41–45.30 |

* Not on hormone therapy

3. Interpret the results of this test in conjunction with the patient's clinical presentation.

## Specific Performance Characteristics

### Methods Comparison

A comparison of 113 values using the Access Progesterone assay on the Access Immunoassay system and a commercially available immunoassay kit gave the following statistical data:

| n | Range of Observations (ng/mL) | Intercept (ng/mL) | Slope | Correlation Coefficient (r) |
|---|---|---|---|---|
| 113 | 0.39–30.41 | 0.63 | 0.824 | 0.978 |

### Dilution Recovery (Linearity)

Multiple dilutions of two samples containing elevated progesterone levels with Access Progesterone Calibrator S0 (zero) resulted in the following data:

| Sample 1 | Expected Concentration (ng/mL) | Determined Concentration (ng/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 22.16 | N/A |
| 1/2 | 11.08 | 11.73 | 106 |
| 1/3 | 7.39 | 8.24 | 112 |
| 1/4 | 5.54 | 6.69 | 121 |
| 1/8 | 2.77 | 3.24 | 117 |
| 1/16 | 1.39 | 1.68 | 121 |
| | | **Mean % Recovery** | **115** |

| Sample 2 | Expected Concentration (ng/mL) | Determined Concentration (ng/mL) | Recovery (%) |
|---|---|---|---|
| Neat | N/A | 24.44 | N/A |
| 1/2 | 12.22 | 12.45 | 102 |
| 1/3 | 8.15 | 8.80 | 108 |
| 1/4 | 6.11 | 6.88 | 113 |
| 1/8 | 3.05 | 3.52 | 115 |
| 1/16 | 1.53 | 1.84 | 121 |
| | | **Mean % Recovery** | **112** |

### Imprecision

One study, using commercially available human serum based control material generating a total of 20 assays, 2 replicates per assay, over 10 days provides the following data, analyzed via analysis of variance (ANOVA).[15,16]

| Human Serum Control | Grand Mean (n=40) (ng/mL) | Within Run (% CV) | Between Run (% CV) | Total Imprecision (% CV) |
|---|---|---|---|---|
| Level 1 | 1.26 | 11.19 | 9.57 | 14.73 |
| Level 2 | 9.42 | 7.25 | 6.59 | 9.80 |
| Level 3 | 22.61 | 6.11 | 7.51 | 9.85 |

### Analytical Specificity/Interferences

Serum samples containing up to 5 mg/dL (85.5 µmol/L) bilirubin, hemolyzed samples containing up to 500 mg/dL (5 g/L) hemoglobin and lipemic samples containing the equivalent of 450 mg/dL (5.08 mmol/L) triglycerides do not affect the concentration of progesterone assayed using an initial sample containing approximately 7 ng/mL progesterone.

The following table describes the cross-reactivity of the assay with substances that are similar in structure to progesterone. Potential cross-reactants were spiked into the S3 calibrator.

| Substance | Analyte Added (ng/mL) | Apparent Concentration (ng/mL) | Cross-Reactivity (%) |
|---|---|---|---|
| 17-α hydroxprogesterone | 50 | 1.18 | 2.36 |
| Pregnenolone | 200 | 0.73 | 0.36 |
| DHEA sulfate | 4000 | ND | ND |
| 5β-pregnane-3α, 20α-diol-3 glucuronide | 200 | ND | ND |
| Cortisol | 600 | 0.46 | 0.08 |
| 11-deoxycortisol | 100 | ND | ND |
| Corticosterone | 15 | 0.91 | 6.08 |
| Androstenediol | 50 | ND | ND |
| 20-α dihydroprogesterone | 100 | 0.66 | 0.66 |
| 17-β estradiol | 10 | ND | ND |
| Estriol | 10 | ND | ND |
| Testosterone | 10 | ND | ND |
| Cortisone | 100 | ND | ND |
| Prednisolone | 200 | ND | ND |
| Medroxprogesterone | 100 | 1.38 | 1.38 |
| Danazol | 100 | ND | ND |

ND = Not detectable

### Analytical Sensitivity

The lowest detectable level of progesterone distinguishable from zero (Access Progesterone Calibrator S0) with 95% confidence is 0.10 ng/mL (0.32 nmol/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 mean measured zero calibrator signal.

# Access
Immunoassay Systems

# PROGESTERONE CALIBRATORS
## REF 33555

**Intended Use**

The Access Progesterone Calibrators are intended to calibrate the Access Progesterone assay for the quantitative determination of progesterone levels in human serum 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 Progesterone Calibrators is traceable to USP reference material. 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 Progesterone Calibrators**
**Cat. No. 33555: S0, 4.0 mL/vial; S1–S5, 2.5 mL/vial**

- Provided ready to use.
- Store at -20°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at -20°C.
- After thawing, calibrators are stable for 3 months 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 0.0 ng/mL (nmol/L) progesterone. |
| **S1, S2, S3, S4, S5:** | Progesterone (purified chemical compound) in human serum at levels of approximately 1.0, 4.0, 10.0, 20.0 and 40.0 ng/mL (3.18, 12.72, 31.80, 63.60, and 127.20 nmol/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.[17]

- 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.[9]
- 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**

**Run the Access Progesterone Calibrator S0 in quadruplicate, the S1 calibrator in triplicate**, and the S2–S5 calibrators in duplicate.

The Access Progesterone Calibrators are provided at six levels – zero and approximately 1.0, 4.0, 10.0, 20.0 and 40.0 ng/mL – prepared gravimetrically from synthetic progesterone and human serum. Assay calibration data are valid up to 28 days.

**Limitations of the Procedure**

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

# Access
Immunoassay Systems

# PROGESTERONE CALIBRATOR S0
## REF 33556

**Intended Use**

The Access Progesterone Calibrator S0 is intended for use with the Access Progesterone 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 Progesterone Calibrator S0**

**Cat. No. 33556: 4 mL/vial**

- Provided ready to use.
- Store at -20°C.
- Mix contents by gently inverting before use. Avoid bubble formation.
- Stable until the expiration date stated on the label when stored at -20°C.
- After thawing, stable for three months when stored at 2 to 10°C.

| | |
|---|---|
| **S0:** | Human serum, < 0.1% sodium azide, and 0.025% Cosmocil** CQ. Contains 0.0 ng/mL (nmol/L) progesterone. |

**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.[17]
- 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.[9]
- 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 the reagent, discard the vial.

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