# 17 α OH Progesterone

**NB-06-0490**

# 17 α OH Progesterone

**#Cat: NB-06-0490 Size: 1x96 Tests**

## 1. Introduction

17-Hydroxyprogesterone (17- OH progesterone or 17OHP) is a C-21 steroid hormone produced in the adrenal gland and gonads, during the synthesis of glucocorticoids and sex steroids. It is derived from progesterone via 17-hydroxylase, a P450c17 enzyme, or from 17-hydroxypregnenolone via 3β-hydroxysteroid dehydrogenase/ 5-4 isomerase.
17α -OHP has no defined physiologic role except as a precursor molecule.
Serum 17α -OHP levels are age-dependent, with peak levels observed during fetal life and the immediate postnatal period. During the first week of life, serum 17α -OHP levels fall ~50-fold as compared to cord blood values. A small transient increase occurs in male infants 30-60 days postnatally. Levels for both sexes remain at constant low levels during childhood, and then progressively increase during puberty reaching adult levels of ~100 ng/dL (~3.03 nmol/l). As with cortisol, serum 17 α -OHP levels normally have an ACTH-dependent diurnal variation, with peak levels in the morning and a nadir at night. In addition, ovarian production of 17α -OHP increases during the luteal phase of the menstrual cycle. 17-hydroxyprogesterone is a natural progestin and in pregnancy increases in the third trimester primarily due to fetal adrenal production.
Normal levels are 3-90 ng/dl in children and in women, 15-70 ng/dl prior to ovulation, and 35-290 ng/dl during the luteal phase.
Measurements of levels of 17-hydroxyprogesterone are useful in the evaluation of patients with suspected congenital adrenal hyperplasia as the typical enzymes that are defective, namely 21-hydroxylase and 11 -hydroxilase, lead to a build-up of 17OHP. In contrast, the rare patient with 17α-hydroxylase deficiency will have very low or undetectable levels of 17OHP.
Elevated serum 17 α -OHP levels at baseline and/or after ACTH stimulation have also been reported in other forms of adrenal hyperplasia.

## 2. Intended use

Competitive immunoenzymatic colorimetric method for quantitative determination of 17 α OH Progesterone in serum or plasma.

## 3. Principle of the assay

Microtiter strip wells are precoated with anti- 17 α OH Progesterone antibodies (solid-phase). 17 α OH Progesterone in the sample competes with added horseradish peroxidase labelled 17 α OH Progesterone (enzyme-labelled antigen) for antibody binding. After incubation a bound/free separation is performed by solid-phase washing. The immune complex formed by enzyme-labelled antigen is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is inversely proportional to the amount of 17 α OH Progesterone in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorption at 450 nm is read using an ELISA microwell plate reader.

## 4. Materials

### 4.1. Reagents supplied

▪ **Anti-17 α OH Progesterone IgG Coated Wells:** 12 break-apart 8-well snap-off strips coated with anti-17 α OH Progesterone IgG; in resealable aluminium foil.

▪ **Stop Solution**: 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).

▪ **17 α OH Progesterone-HRP Conjugate:** 1 bottle containing 6 ml of horseradish peroxidase labelled 17 α OH Progesterone.

▪ **TMB Substrate Solution**: 1 bottle containing 12 ml 3, 3´, 5, 5´-tetramethylbenzidine ($H_2O_2$-TMB 0.26 g/l) (avoid any skin contact).

▪ **17 α OH Progesterone Standards:** 6 bottles, 1 ml each

| | |
|---|---|
| Standard 0: | 0 ng/ml |
| Standard 1: | 0.2 ng/ml |
| Standard 2: | 0.4 ng/ml |
| Standard 3: | 1.6 ng/ml |
| Standard 4: | 6.4 ng/ml |
| Standard 5 | 19.2 ng/ml |

### 4.2. Materials supplied

▪ 1 Strip holder
▪ 1 Cover foil
▪ 1 Test protocol
▪ 1 Distribution and identification plan

### 4.3. Materials and Equipment needed

▪ ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
▪ Manual or automatic equipment for rinsing wells
▪ Pipettes to deliver volumes between 10 and 1000 µl
▪ Vortex tube mixer
▪ Distilled water
▪ Disposable tubes
▪ Timer

## 5. Stability and storage

The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.

## 6. Reagent preparation

It is very important to bring all reagents, samples and standards to room temperature (22…28°C) before starting the test run!

### 6.1. Coated snap-off Strips

The ready to use break apart snap-off strips are coated with anti-17 α OH Progesterone IgG antibodies. Store at 2…8 °C. Open the bag only when it is at room temperature. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.

### 6.2. 17 α OH Progesterone -HRP Conjugate

17 α OH Progesterone-HRP Conjugate is a ready to use solution.

### 6.3. 17 α OH Progesterone Standards

The standards are ready to use and have the following concentration of 17 α OH Progesterone:

| | |
|---|---|
| Standard 0: | 0 ng/ml |
| Standard 1: | 0.2 ng/ml |
| Standard 2: | 0.4 ng/ml |
| Standard 3: | 1.6 ng/ml |
| Standard 4: | 6.4 ng/ml |
| Standard 5 | 19.2 ng/ml |

After first opening stable for another 6 months at 4°C.

### 6.4. TMB Substrate Solution

The bottle contains 12 ml of a tetramethyl benzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. The solution should be colour less or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

### 6.5. Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

## 7. Specimen collection and preparation

The determination of 17 α OH Progesterone can be performed in plasma as well as in serum. Store the sample at -20°C if the determination is not performed on the same day as the sample collection. If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

## 8. Assay procedure

### 8.1. Test Preparation

Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the

distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

| | | |
|---|---|---|
| 1 well | (e.g. A1) | for the substrate blank |
| 2 wells | (e.g. B1+C1) | for standard 0 |
| 2 wells | (e.g. D1+E1) | for standard 1 |
| 2 wells | (e.g. F1+G1) | for standard 2 |
| 2 wells | (e.g. H1+A2) | for standard 3 |
| 2 wells | (e.g. B2+C2) | for standard 4 |
| 2 wells | (e.g. D2+E2) | for standard 5 |

It is necessary to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 50 µl standards and samples into their respective wells. Leave well A1 for substrate blank.
2. Add 50 µl 17 α OH Progesterone-HRP Conjugate to each well. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. Incubate for 1 hour at 37 °C.
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well twice with 300 µl distilled water. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
6. Dispense 100 µl TMB Substrate Solution into all wells.
7. Incubate for exactly 15 min at room temperature (22…28°C) in the dark.
8. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
   Any blue colour developed during the incubation turns into yellow.
9. Measure the absorbance of the specimen at 450 nm within 30 min after addition of the Stop Solution.

### 8.2. Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard and patient sample in the distribution and identification plan.
Where applicable calculate the mean absorbance values of all duplicates.

## 9. Results

### 9.1. Calculation of results

Calculate the mean absorbance for each point of the standard curve and each sample. Plot the mean value of absorbance of the standards against concentration. Draw the best-fit curve through the plotted points. (Four Parameter Logistic).
Interpolate the values of the samples on the standard curve to obtain the corresponding values of the concentrations expressed in ng/ml.

### 9.2. Reference values

The serum or plasma 17 α OH Progesterone reference values are:

| | | |
|---|---|---|
| Children | | 0.2 – 0.9 ng/ml |
| Men | | 0.2 – 2.3 ng/ml |
| Women | follicular phase | 0.2 – 1.3 ng/ml |
| | luteinic phase | 1.0 – 4.5 ng/ml |
| | Menopause | 0.2 – 0.9 ng/ml |

### 10. Quality control

Each laboratory should assay controls at normal, high and low levels range of 17OH Progesterone for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the standard curve for run-to-run reproducibility. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.
If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

### 11. Specific performance characteristics

### 11.1. Precision

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is ≤ 7.4%.

Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in different lots. The between assay variability is ≤ 13%.

### 11.2. Specificity

The cross reaction of the antibody calculated at 50% according to Abraham is:

| | |
|---|---|
| 17 OH Progesterone | 100.0 % |
| 17 OH Pregnenolone | 1.3 % |
| Progesterone | 1.2 % |
| Cortisol | 0.02 % |
| Cholesterol | $8 \times 10^{-4}$ % |

### 11.3. Sensitivity

The lowest detectable concentration of 17 OH progesterone that can be distinguished from the zero standard is 0.09 ng/ml at the 95 % confidence limit.

### 11.4. Accuracy

The recovery of 1 – 2 - 4 ng/ml 17 α OH Progesterone added to a sample gave an average value (±SE) of 103.94% ± 2.78% with reference to the original concentrations.

### 11.5. Method comparison

The GenWay 17 OH Progesterone ELISA was compared to another commercially available 17OH progesterone assay (RIA). 38 serum samples were analysed according in both test systems.

(17 OH-Progesterone GenWay)=0.83*(17 OH Progesterone RIA) + 0.17 $r^2$ = 0.946

## 12. Limitations of the procedure

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

## 13. Precautions and warnings

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to

be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.

- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.
- Do not use heavily haemolysed or highly lipemic samples.
- Maximum precision is required for dispensation of the reagents.
- Avoid the exposure of TMB substrate to direct sunlight, metal or oxidants.
- This method allows the determination of 17 OH Progesterone from 0.2 – 19.2 ng/ml.
- Treatment of the patient with cortisone, natural or synthetic steroids can impair 17 α OH Progesterone determination.
- The reagents contain Proclin 300® as preservative.

| | |
|---|---|
| WARNING: | Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor! |

### 13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste

**SCHEME OF THE ASSAY**

17 α OH Progesterone

**Test Preparation**

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

**Assay Procedure**

| | Substrate blank | Standard 0 | Standard 1 | Standard 2 | Standard 3 | Standard 4 | Sample |
|---|---|---|---|---|---|---|---|
| Standard 0 | - | 50 µl | - | - | - | - | - |
| Standard 1 | - | - | 50 µl | - | - | - | - |
| Standard 2 | - | - | - | 50 µl | - | - | - |
| Standard 3 | - | - | - | - | 50 µl | - | - |
| Standard 4 | - | - | - | - | - | 50 µl | - |
| Sample | - | - | - | - | - | - | 50 µl |
| Conjugate | - | 50 µl | 50 µl | 50 µl | 50 µl | 50 µl | 50 µl |
| Cover wells with foil supplied in the kit<br>**Incubate for 1 h at 37 °C**<br>Wash each well twice with 300 µl distilled water | | | | | | | |
| TMB Substrate | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| **Incubate for exactly 15 min at room temperature in the dark** | | | | | | | |
| Stop Solution | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| Photometric measurement at 450 nm | | | | | | | |



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