

 **Labor Diagnostika Nord GmbH & Co. KG**

Am Eichenhain 1, 48531 Nordhorn

Telefon: +49-5921-8197 0

Telefax: +49-5921-8197 222

e-mail: [manz@ldn.de](mailto:manz@ldn.de)

Internet: <http://www.ldn.de>

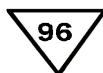


---

**Instructions for use**  
**17-OH-Progesterone ELISA**

**REF**

**FR E-2800**



**IVD**



## **17-OH-Progesterone ELISA**

### **1. Intended use and principle of the test**

Enzyme Immunoassay for the direct quantitative determination of 17-OH-Progesterone (17-OHP) in human serum

The principle of the following enzyme immunoassay test follows the typical competitive binding scenario. Competition occurs between an unlabeled antigen (present in standards, control and patient samples) and an enzyme-labelled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. The washing and decanting procedures remove unbound materials. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed is inversely proportional to the concentration of 17 $\alpha$ -OHP in the sample. A set of standards is used to plot a standard curve from which the amount of 17 $\alpha$ -OHP in patient samples and controls can be directly read.

### **2. Procedural cautions and warnings**

This kit is intended for in vitro use only.

Practice the following good laboratory practices when handling kit reagents:

- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
- Wash hands thoroughly after performing the test.
- Avoid contact with eyes; use safety glasses; in case of contact, flush with water immediately and contact a doctor.
- Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Avoid microbial contamination of reagents.
- A Standard curve must be established for every run.
- It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
- The controls (included in kit) should be included in every run and fall within established confidence limits.
- When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
- When reading the microplate, the presence of bubbles in the microwells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
- The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
- When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
- Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

### **3. Limitations**

- Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
- Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
- Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
- The results obtained with this kit should never be used as the sole basis for a clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.

#### 4. **Safety cautions and warnings**

POTENTIAL BIOHAZARDOUS MATERIAL

All serum samples should be considered a potential biohazard and handled with the appropriate precautions.

##### **Chemical hazards**

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

#### 5. **Sample collection and storage**

Approximately 0.1 ml of serum is required per duplicate determination.

Collect 4-5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer.

Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

#### 6. **Additional materials and equipment required but not provided in the kit**

- Precision pipettes to dispense 25, 50, 100, 150 and 300 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater (see test procedure step 11).

#### 7. **Reagents provided**

The 17-OH-Progesterone ELISA kit FR E-2800 contains enough reagents for 96 quantitative determinations.

##### **96 FR E-2831 Rabbit Anti-17α-OHP Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use Break Apart Wells.**

Contents: One 96 well (12x8) polyclonal antibody-coated microwell plate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

##### **CONJUGATE-CONC 100x FR E-2840 17α-OHP-Horseradish Peroxidase (HRP) Conjugate Concentrate - X100**

Contents: 17-OHP-HRP conjugate in a protein-based buffer with a non-mercury preservative.

Volume: 300 µl/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:100 in assay buffer before use (eg. 20 µl of HRP in 2 ml of assay buffer). If the whole plate is to be used dilute 180 µl of HRP in 18 ml of assay buffer. Discard any that is left over.

##### **17α-OHP Calibrators - Ready To Use**

Contents: Seven vials containing 17-OHP in a human serum-based buffer with a non-mercury preservative. Prepared by spiking serum with a precise quantity of 17-OHP.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

	<b>Catalogue no.</b>	<b>Standard</b>	<b>Concentration</b>	<b>Volume/Vial</b>
<b>STANDARD A</b>	<b>FR E-2801</b>	Standard A	0 ng/mL	2.0 mL
<b>STANDARD B</b>	<b>FR E-2802</b>	Standard B	0.15 ng/mL	0.6 mL
<b>STANDARD C</b>	<b>FR E-2803</b>	Standard C	0.5 ng/mL	0.6 mL
<b>STANDARD D</b>	<b>FR E-2804</b>	Standard D	1.5 ng/mL	0.6 mL
<b>STANDARD E</b>	<b>FR E-2805</b>	Standard E	3 ng/mL	0.6 mL
<b>STANDARD F</b>	<b>FR E-2805</b>	Standard F	7.5 ng/mL	0.6 mL
<b>STANDARD G</b>	<b>FR E-2806</b>	Standard G	20 ng/mL	0.6 mL

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

**CONTROL****FR E-2851/FR E-2852 Controls - Ready To Use**

Contents: Two vials containing 17 $\alpha$ -OHP in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of 17 $\alpha$ -OHP. Refer to vial label for expected value and acceptable range.

Volume: 0.6 mL/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months in unopened vial or as indicated on label. Once opened, the control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

**WASH-CONC 10x****AA E-0030 Wash Buffer Concentrate - X10**

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.

Volume: 50 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water.

**ASSAY-BUFF****FR E-2813 Assay Buffer - Ready To Use**

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 20 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

**SUBSTRATE****AA E-0055 TMB Substrate - Ready To Use**

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.

Volume: 16 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

**STOP-SOLN****AA E-0080 Stopping Solution - Ready To Use**

Contents: One vial containing 1M sulfuric acid.

Volume: 6 ml/vial

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

**8. Test procedure**

All reagents must reach room temperature before use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

<b>1.</b>	Prepare <b>working solutions</b> of the 17 $\alpha$ -OHP-HRP conjugate and wash buffer.
<b>2.</b>	Remove the required number of microwell strips. Reseal the bag and return any unused strips to the refrigerator.
<b>3.</b>	Pipette <b>50 <math>\mu</math>L</b> of each standard, control and specimen sample into the corresponding wells in duplicate.
<b>4.</b>	Pipette <b>150 <math>\mu</math>L</b> of the conjugate working solution into each well.
<b>5.</b>	Incubate on a plate <b>shaker</b> (approximately 200 rpm) for <b>1 hour at room temperature</b> .
<b>6.</b>	Wash the wells <b>3 times</b> with prepared wash buffer (300 $\mu$ L/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
<b>7.</b>	Pipette <b>150 <math>\mu</math>L</b> of <b>TMB substrate</b> into each well at timed intervals.
<b>8.</b>	Incubate on a plate shaker for 10-15 minutes at room temperature (or until calibrator A attains dark blue colour for desired OD).
<b>9.</b>	Pipette <b>50 <math>\mu</math>L</b> of <b>stopping solution</b> into each well at the same timed intervals as in step 8.
<b>10.</b>	Read the plate on a microwell plate reader at <b>450 nm</b> within 20 minutes after addition of the stopping solution.  If the OD exceeds the upper limit of detection or if a 450nm filter is unavailable, a 405 or 415nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

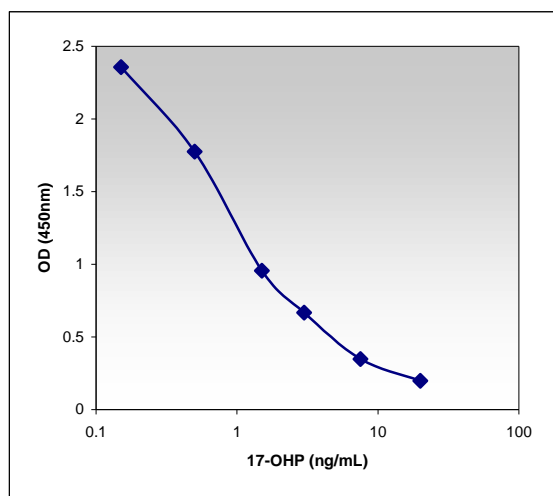
## 9. Calculation of results

1. Calculate the mean optical density of each Standard duplicate.
2. Draw a Standard curve on semi-log paper with the mean optical densities on the Y-axis and the Standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the Standard curve.
5. If a sample reads more than 20 ng/mL then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

**Typical tabulated data** (example, do not use for your own calculation)

Calibrator	OD 1	OD 2	Mean OD	Value (ng/mL)
A	2.531	2.558	2.544	0
B	2.357	2.326	2.341	0.15
C	1.776	1.749	1.762	0.5
D	0.957	1.016	0.986	1.5
E	0.668	0.732	0.700	3
F	0.348	0.360	0.354	7.5
G	0.198	0.201	0.199	20
Unknown	0.823	0.825	0.824	2.15

**Typical Standard curve**\_(example, do not use for your own calculation)



## 10. Assay characteristics

### **SENSITIVITY**

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of calibrator A (based on 10 replicate analyses) minus 2 SD. Therefore, the sensitivity of this 17 $\alpha$ -OHP ELISA kit is 0.11 ng/mL.

### SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with the 17 $\alpha$ -OHP ELISA kit with 17 $\alpha$ -OHP cross-reacting at 100%.

Steroid	%Cross-Reactivity
17 $\alpha$ -Hydroxy-progesterone	100
Progesterone	0.25
11-Desoxycortisol	0.77
DHEA	<0.1
Corticosterone	<0.1
Cholesterol	<0.1
Pregnenolone	<0.1
Pregnenolone-SO <sub>4</sub>	<0.1
Prednisone	<0.1

### INTRA-ASSAY PRECISION

Three samples were assayed 20 times, on the same calibrator curve. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	1.91	0.096	5.0
2	5.68	0.287	5.1
3	10.48	0.602	5.7

### INTER-ASSAY PRECISION

Three samples were assayed 10 times over a period of four weeks. The results (in ng/mL) are tabulated below:

Sample	Mean	SD	CV%
1	1.69	0.116	6.8
2	5.69	0.487	8.6
3	9.38	0.464	4.9

### RECOVERY

Spiked samples were prepared by adding defined amounts of 17 $\alpha$ -OHP to three patient serum samples. The results (in ng/mL) are tabulated below:

Sample	Obs.Result	Exp.Result	Recovery%
1	1.82	-	-
0.15*	2.61	2.75	95
0.4*	4.66	4.8	97
1.6*	13.81	14.8	93
2	2.79	-	-
0.15*	3.57	3.57	100
0.4*	5.90	5.66	104
1.6*	15.47	15.66	99
3	3.70	-	-
0.15*	4.29	4.3	100
0.4*	5.942	6.4	93
1.6*	16.49	16.4	101

\* - Amount added (ng/0.1 mL)

**LINEARITY**

Three patient serum samples were diluted with calibrator A. The results (in ng/mL) are tabulated below:

Sample	Obs.Result	Recovery%
1	3.52	-
1:2	1.50	86
1:4	0.88	101
1:8	0.46	107
2	5.47	-
1:2	2.7	99
1:4	1.21	89
1:8	0.66	97
3	18.29	-
1:2	8.75	96
1:4	4.54	99
1:8	2.27	100







**EXPECTED NORMAL VALUES**

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (ng/mL)
Newborn	
5-30 day	<0.7-2.5
31-60 day male	0.8-5.0
31-60 day female	0.5-2.3
Children	
3-14 years	0.07-1.7
Females:	
Follicular phase	0.2-1.3
Luteal phase	1.0-4.5
Postmenopausal	0.2-0.9

 **Actual literature, information about clinical significance or any other information about the test are available on the homepage or contact the manufacturer directly.**

**Symbols:**

 +2/+8 °C	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	<b>LOT</b>	Batch code	<b>IVD</b>	For in-vitro diagnostic use only!
	Consult instructions for use	<b>CONT</b>	Content	<b>CE</b>	CE labelled
	Caution	<b>REF</b>	Catalogue number	<b>RUO</b>	For research use only!