



# ELEGANCE PROGESTERONE ELISA KIT

REF 40 490096

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## WARRANTY

The manufacturer makes no express warranty other than the diagnostic kit will measure the designated analyte when used in accordance with the manufacturer's printed instructions. The use of the diagnostic kit for any other purpose is outside the intended use of this product and is done at the user's own risk.

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## INTENDED USE

The ELEGANCE Progesterone ELISA has been designed for the quantitative *in vitro* diagnostic measurement of Progesterone (P<sub>4</sub>) in serum or plasma.

## PRINCIPLES OF THE ELEGANCE ELISA

The ELEGANCE P<sub>4</sub> ELISA is an enzyme-linked immunoassay incorporating an anti-progesterone monoclonal antibody (Antibody Reagent) and an anti-mouse IgG polyclonal antibody bound to the microwells. It is a one-step "competitive" method, utilising a progesterone-horse radish peroxidase conjugate (P<sub>4</sub>-HRP) to produce the signal generated. During the incubation complexes are formed between the monoclonal antibody and sample antigen or P<sub>4</sub>-HRP. This occurs as the antibody, itself is captured by the microwell bound secondary antibody. The microwells are washed to remove any unbound material. After washing the substrate solution reacts with any bound peroxidase to produce colour inversely proportional to the amount of sample antigen, which can be calculated from the calibrator curve.

## ELEGANCE REAGENTS PROVIDED, STABILITY AND STORAGE

Kit size - 96 tests. The kit and all its components, unopened or opened, should be stored at 2-8°C until the listed expiry dates.

**Progesterone: Coated Microwells**  
**96 wells REF # P4A96**  
Frame containing microwells coated with anti-murine IgG antibody. Ready to use.

**Progesterone: Antibody Reagent**  
**1 vial REF # P4B96**  
5 mL murine anti-progesterone antibody in a buffered solution containing animal serum proteins containing a blue dye. Contains Bronidox L, 0.2% v/v and thiomersal, 0.01% w/v. Ready to use.

**Progesterone: Conjugate Reagent**  
**1 vial REF # P4C96**  
5 mL progesterone-HRP conjugate in a buffered solution containing a violet dye. Contains Bronidox L, 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

**Wash Concentrate**  
**1 vial REF # EWC96**  
50 mL of a 15 x concentrated wash solution. Contains thiomersal, 0.09% w/v. To be diluted before use.

## Substrate Solution

**TMB N**  
**1 vial REF # TMBB96**  
10 mL containing 3,3',5,5' - tetramethylbenzine (TMB N) and hydrogen peroxide in a stabilizing solution. Ready to use.

**Progesterone: Calibrators**  
**6 vials REF # EP4S1-6**  
2.0 mL in Calibrator A and 0.5 mL in Calibrator B-F, each in human serum. Contains thiomersal, 0.01% w/v. Lyophilized.

## PRECAUTIONS AND

### WARNINGS TO USERS

Handling of specimens and kit components, their use, storage and disposal should be in accordance with any local or national laboratory safety procedures or regulations.

### Specimens and Calibrators

The source material of the calibrators has been tested by an approved accredited method for the presence of hepatitis B surface antigen, antibody to hepatitis C and antibody to HIV - 1/2 (AIDS) and has been found to be non-reactive for all. However it is recommended that all samples be handled as if capable of transmitting infectious disease.

### Preservatives

The kit contains thiomersal and Bronidox L as preservatives. As reagents contain potentially toxic preservatives, care should be taken in handling, to avoid ingestion or skin contact.

### Substrate

Avoid any skin contact.

## SPECIMEN COLLECTION AND HANDLING

No special patient preparation is required. Specimens can be either serum or plasma collected in a manner appropriate for laboratory testing. Serum is preferred, however the anticoagulants heparin or EDTA can be employed without sacrificing accuracy.

Avoid grossly haemolytic, lipaemic and turbid specimens. Specimens can be stored at 2-8°C for up to 48 hours. Specimens held for longer should be stored at or below -20°C. Specimens should not be frozen and thawed repeatedly. Thawed specimens should be checked for flocculent matter and mixed by inversion just prior to testing.

Turbid specimens or specimens containing particulate matter should be centrifuged prior to use.

## MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- \* Distilled or deionised water
- \* 1M H<sub>2</sub>SO<sub>4</sub>
- \* Precision pipettes
- \* Repeating pipette
- \* 1L measuring cylinder
- \* Absorbent tissue (lint-free)
- \* Timer
- \* Vortex mixer
- \* Microtitre plate shaker
- \* Microtitre plate washer
- \* Microplate reader system.

## PROCEDURAL NOTES

Bring all reagents and specimens to room temperature (20-25°C) and mix by gentle inversion prior to use. Duplicates are recommended. Contamination of reagents will lead to poor performance. A calibrator curve should be run with each assay. Specimens suspected of having concentrations above the top calibrator should be diluted in zero calibrator before assay. All assay steps should be performed without interruption, but if the wells cannot be filled with Conjugate Reagent or Substrate Solution immediately after washing, then the microwells may be left upside down on absorbent lint-free tissue for a maximum of 15 minutes.

Reagents are matched in each kit and therefore reagents from different lot numbers should not be mixed.

The photometer and all pipettes used should be calibrated appropriately before use.

### Washing

The efficiency of the wash step is vital for good precision. Microwells are washed using an automatic plate washer. Avoid overflows from one well to another.

### Quality Control

Control specimens should be run in every assay to ensure correct procedure. Control values should lie within laboratory ranges before assay is approved.

## ASSAY PROCEDURE

### Preparation of Reagents

#### Wash Solution

Dilute the wash concentrate 1 in 15 with deionised water. The wash solution can be stored at room temperature (20-25°C) for 12 weeks.

#### Calibrators

To reconstitute the lyophilized calibrators, add the volume of deionized water indicated on each vial label. Allow the vials to sit undisturbed until completely dissolved (at least 30 minutes) and then mix by gentle inversion.

Exact concentrations determined lot-to-lot are stated on a separate label inside the kit.

After reconstitution, the calibrators should be stored at -20°C for up to 4 weeks.

#### Protocol

1. Assemble the microwells in the frame according to the number of tests required. Bag and return unused wells to 2-8°C.

2. Pipette 50 µL of sample (calibrator, control, specimen) in duplicate into the appropriate wells. Time taken to dispense the samples should not exceed 40 minutes.

3. Pipette 50 µL of Progesterone Conjugate Reagent (violet) into all wells.

4. Pipette 50 µL of Progesterone Antibody Reagent (blue) into all wells.

5. Cover microwells with lid and incubate for 60 minutes on a plate shaker at room temperature (20-25°C).

6. After incubation, wash the microwells. Aspirate the liquid and rinse each well 4 times with 250 µL wash solution. After the final wash, invert the microwells and tap firmly on absorbent tissue to remove any remaining wash solution. Ensure that no air bubbles remain in the wells.

7. Pipette 100 µL of Substrate Solution (TMB N) into all wells. Timing of the incubation step is measured from the addition of Substrate Solution to the first well.

8. Cover microwells with lid and incubate for 5 minutes stationary at room temperature (20-25°C).

9. Pipette 50 µL of 1M H<sub>2</sub>SO<sub>4</sub> into all wells in the same timed sequence as for Substrate Solution addition.

10. Carry out an end-point reading at 450 nm and process data as described in the microplate reader user's manual. This reading step should be carried out within 15 minutes of stopping the reaction.

#### EXPECTED VALUES

It is recommended that each laboratory establish its own reference range based on a representative sample population. The following reference range calculated at a 95% confidence interval, was obtained by assaying serum samples from healthy individuals and is given as a guide only:

	n	Range (nmol/L)	
Adult Male	68	< 4.5	(2.2)
Adult Female			
Follicular Phase	36	0.7 - 3.5	(2.2)
Luteal Phase	40	>> 5.0*	
Post-menopausal	11	< 1.6	(0.7)

\* generally <60 nmol/L; () bracketed numbers are mean

#### During pregnancy

1st trimester	< 100	nmol/L
2nd trimester	100 - 300	nmol/L
3rd trimester	> 300	nmol/L

#### CALCULATION OF RESULTS

Calculation of results can be carried out manually if there is no automatic data reduction. Determine the OD for each well. Plot the calibrator curve using log-log graph paper with concentration of calibrators on the x-axis and OD on the y-axis. The curve may be drawn point-to-point or a curve-fitting routine, such as 4-parameter logistic interpolation, may be used. Interpolate the sample values from OD measured from this calibrator curve.

Record the value for each sample in nmol/L P<sub>4</sub>. The range of the *ELEGANCE* Progesterone ELISA is from 0 to approx. 100 nmol/L, but the minimum concentration that can be reported is limited by the linear performance characteristics of the photometer used. If the OD value of the zero or lowest calibrator is above the range of the photometer, then this calibrator must be omitted from the plot of the calibrator curve.

#### MODEL CALCULATIONS

Endpoint Data		
ID	Ave OD	P <sub>4</sub> (nmol/L)
0	2.815	
1.0	2.230	
3.0	1.723	
10	0.867	
30	0.367	
100	0.128	
Sample 1	1.523	3.79
Sample 2	0.457	23.40
Sample 3	0.224	53.30

#### CALIBRATION

The calibrators supplied in this kit are calibrated and labelled in nmol/L. Conversion of the calibrator units may be made using the following relationship:

$$\text{ng/mL P}_4 = \frac{\text{nmol/L P}_4}{3.18}$$

#### LIMITATIONS

Serum specimens showing gross haemolysis, gross lipaemia, or turbidity may give false results.

#### PERFORMANCE CHARACTERISTICS

##### Intra-assay Precision

Sample	n	Mean ± 2SD (nmol/L)	%CV
1	16	3.35 ± 0.34	5.1
2	16	18.50 ± 0.91	2.5
3	16	41.40 ± 2.62	3.2

##### Inter-assay Precision

Sample	n *	Mean ± 2SD (nmol/L)	%CV
1	40	3.40 ± 0.59	8.7
2	40	19.90 ± 2.87	7.2
3	40	46.40 ± 6.79	7.3

\* duplicate

##### Accuracy

Recovery was calculated by assaying before and after addition of exogenous analyte.

Sample	P <sub>4</sub> (nmol/L) Observed	P <sub>4</sub> (nmol/L) Expected	% Recovery
1	4.64	4.77	97.3
2	5.36	5.12	104.6
3	5.79	6.12	94.6
4	8.59	9.62	89.3

##### Dilution

A sample was diluted in zero calibrator, assayed and recovery calculated.

Sample	P <sub>4</sub> (nmol/L) Observed	P <sub>4</sub> (nmol/L) Expected	% Recovery
Neat	21.0		
1/2	10.4	10.5	99.0
1/4	5.1	5.3	97.1
1/8	2.5	2.6	96.2

##### Specificity

Analyte	%Crossreactivity
Progesterone*	100.00
17 α-OH-Progesterone	1.80
Pregnenolone	1.10
Deoxycorticosterone	0.47
Androstenediol	<0.20
Cholesterol	<0.20
Corticosterone	>0.20
Cortisol	<0.20
11-Deoxycortisol	<0.20
17α-Oestradiol	<0.20
17β-Oestradiol	<0.20
Oestriol	<0.20
Oestrone	<0.20
Pregnanolone	<0.20
20α-OH-Progesterone	<0.20
20β-OH-Progesterone	<0.20
Testosterone	<0.20

##### Sensitivity

The sensitivity of the assay is typically less than 0.25 nmol/L.

The sensitivity is defined as that concentration of analyte which corresponds to the dose response variable (OD) that is two standard deviations from the mean dose response variable of 20 replicate determinations of the zero calibrator run in three different assays.

##### Interference

No interference with analyte recovery was observed for concentrations of haemoglobin up to 250 mg/dL, bilirubin up to 10 mg/dL and triglycerides up to 970 mg/dL.

#### ORDERING INFORMATION

The *ELEGANCE* Progesterone ELISA is manufactured by:

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#### TECHNICAL SERVICE

Full technical service is available by calling Bioclone on +61 (0) 2 9517 1966 or Freecall 1800 251 138

PART No.: *EKBP4 Ed. 5*

Revision date: 31 May 2009