

PROGESTERONE [¹²⁵I] RIA KIT (Ref: RK-460M)

Description

The PROGESTERONE [¹²⁵I] assay system provides the quantitative in vitro determination of progesterone in human serum or plasma. Progesterone can be assayed in the range of 0-120 nmol/l (0-37.7 ng/ml) using 50 µl serum samples. Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and assay of 41 unknowns and 1 control in duplicate.

Introduction

Progesterone is one of the C₂₁-steroids (Mw=314.5) secreted by the corpus luteum in females during the menstrual cycle, and in a much higher amount by the placenta during pregnancy. It is also secreted in a minor quantity by the adrenal cortex in both males and females. Majority of circulating progesterone is bound to albumin and corticosteroid binding globulin (CBG), the bioactive free hormone represents only 2.5-3 % of the total progesterone. Measurement of serum progesterone is of diagnostic value in menstrual disorders and infertility. Measurement of progesterone in the first 10 weeks of gestation, have been suggested in the diagnostic and treatment of patients with threatened abortion and ectopic pregnancy.

Principle of the method

This assay is based on the competition between unlabelled progesterone and a fixed quantity of ¹²⁵I-labelled progesterone for a limited number of binding sites on progesterone specific antibody. Allowing to react a fixed amount of tracer and antibody with different amounts of unlabelled ligand the amount of tracer bound by the antibody will be inversely proportional to the concentration of unlabelled ligand. Upon addition of magnetizable immunosorbent the antigen-antibody complex is bound on solid particles which are then separated by either magnetic sedimentation or centrifugation. Counting the radioactivity of solid phase enables a standard curve to be constructed and samples to be quantitated.

Contents of the kit

1 vial	¹²⁵ I-TRACER, Ready to use. 11 ml per vial, containing about 130 kBq progesterone-11-hemisuccinate- ^[125I] TME in buffer with 0.1% NaN ₃
6 vials	STANDARDS ₁₋₆ , Ready to use. S ₁ = 1 ml, S ₂₋₆ = 0.5 ml per vial, containing 0, 1.5, 4, 12, 40, 120 nmol/l in serum with 0.1% NaN ₃

1 vial	ANTISERUM, Ready to use. 11 ml per vial, containing polyclonal anti-progesterone (rabbit) IgG in buffer with 0.1 % NaN ₃
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1 vial	CONTROL SERUM, Ready to use. 0.5 ml human serum with 0.1% NaN ₃ The concentration of the serum is specified in the quality certificate enclosed.
1 bottle	MAGNETIC IMMUNOSORBENT (MIS). Ready to use. 55 ml per bottle, containing paramagnetic particles in buffer with 0.1 % NaN ₃ Quality certificate. Pack leaflet.

Materials and equipment required

Round bottom polystyrene or polypropylene assay tubes (about 12 x 75 mm), plastic film to cover tubes, precision pipettes (50, 100 µl and 500 µl), vortex mixer, magnetic separator (or alternatively, centrifuge), decanting racks, Gamma counter

Recommended tools and equipment

orbital shaker, repeating pipettes

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Sera can be stored at 2-8°C for two days after collection. For later analysis they should be stored deep-frozen. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens. Samples with a progesterone concentration higher than that of the most concentrated standard should be diluted and reassayed. Use the zero standard as diluent.

Assay procedure

(For a quick guide refer to Table 1)

- 1) Equilibrate all reagents to room temperature.
- 2) Label duplicate tubes for total counts (T), non-specific binding (NSB) zero standard (Standard 1 = B₀), standards (S₂₋₆), control (C) and samples (S_x).
- 3) Mix all reagents and samples thoroughly before use. Avoid excessive foaming.
- 4) Pipette 50 µl each of standards, control and samples into the properly labelled tubes.
- 5) Pipette 100 µl of tracer solution into all tubes.
- 6) Pipette 100 µl of antiserum into all tubes except T and NSB.
- 7) Thoroughly vortex mix all tubes except T for 2-5 seconds. When having an orbital shaker, leave all tubes in the rack holder, fix the holder onto the plate of the shaker, and shake it gently for a few seconds.
- 8) Incubate the tubes for 2 hours at room

	temperature (20-28°C).
9)	Place T tubes on a separate tube rack. Gently shake and swirl the bottle containing magnetic immunosorbent until homogeneity. Add 500 µl to each tube except T. When using a single pipette, swirl the bottle of MIS after every 15-20 tubes. With the use of a repeating pipette (e.g. Eppendorf), there is no need for repeated homogenisation of MIS reagent.
10)	Thoroughly vortex mix all tubes and incubate them for 15 minutes at room temperature.
11)	Separate the bound fraction by using one of the following procedures. Magnetic separation Attach the rack on to the magnetic separator base and ensure that every tube is in contact with the base plate. Let the MIS particles settle for 5 minutes. Do not remove the rack from the separator base after the separation of the solid and liquid phases. Pour off and discard the supernatant. Keeping the separator inverted, place the tubes on a pad of absorbent tissue and allow to drain for 2 minutes. Centrifugation Centrifuge all tubes for 15 minutes at 1500xg or greater. Aspirate the supernatant taking care to avoid disturbing the precipitate.
12)	Count the radioactivity of all tubes preferably not less than 60 seconds.
13)	Calculate the concentrations as described under <i>Calculation of results</i> .

Table 1. Assay Protocol, Pipetting Guide (all volumes in microliters). T=total count, S₁₋₆ =standards, S_xsample, C=control, NSB= Non-specific binding

Tubes	T	NSB	S ₁₋₆	S _x	C
Reagent					
Standard			50		
Sample				50	
Control					50
Tracer	100	100	100	100	100
Anti-serum			100	100	100
Vortex mix. Incubate for 2 hours at room temperature					
Magnetic Immunosorbent		500	500	500	500
Vortex mix. Incubate for 15 minutes at room temperature					
Place the tubes on the magnetic separator for 5 minutes or centrifuge for 15 minutes at 1500xg					

Remove the supernatant and blot the tubes
Count all tubes

Calculation of results

The assay data collected should be similar to those shown in Table 2. Calculate the average counts per minute (CPM) for each pair of assay tubes. Calculate the percent B₀/T for zero standard (S₁) by using the following equation:

$$B_0/T \% = 100 * (S_1 - NSB) / T$$

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/B_0 \% = 100 * (S_{2-6} ; C ; M_x - NSB) / (S_1 - NSB)$$

Using semi-logarithmic graph paper plot B/B₀% for each standard versus the corresponding concentration of progesterone. Figure 1 shows a typical standard curve. Determine the progesterone concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

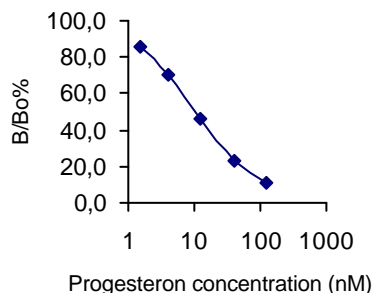
Table 2. Typical Assay Data

Tubes	Counts CPM1	Counts CPM2	AVG CPM	B/T %	B/Bo %
T	45230	46214	45722		
S1	18517	18728	18623	38,7	100,0
S2	16281	16068	16175	33,4	86,2
S3	13515	13284	13400	27,3	70,5
S4	9294	8980	9137	18,0	46,4
S5	5003	4844	4924	8,8	22,6
S6	2694	2880	2787	4,1	10,6
C	5858	5790	5824	10,7	27,7
NSB	869	962	915,5	2,0	

Figure 1.

A typical standard curve

(Do not use to calculate sample values)



Conversion of SI units can be performed according to the following formula:

$$1 \text{ nmol/l} = 0.3145 \text{ ng/ml}$$

$$1 \text{ ng/ml} = 3.18 \text{ nmol/l}$$

Characterization of the assay

Assay parameters

$$NSB/T$$

$$< 3 \%$$

B ₀ /T	47 ± 7 %
ED-50	14 ± 6 nmol/l

Specificity

Cross reactivity was defined by weight at the 50% displacement level in per cent.

Analyte	Cross Reactivity %
Progesterone	100
17-α-Hydroxyprogesterone	13
Pregnenolone	0,03
Cortisol	0,07
Corticosterone	0,6
11-Dezoxi-17-hydroxycorticosterone	0,08
Testosterone	0,01
5-α-Dihydrotestosterone	< 0,001
Estriol	0,003
17-α-Estradiol	< 0,001
Dehydroepiandrosterone	< 0,001

Sensitivity

0.44 ± 0.12 nmol/l, defined as the concentration 2 standard deviations from the zero standard.

Precision, reproducibility

Intra-assay (1 assay in 9 rep.)		Inter-assay (9 assays in 2 rep.)	
Mean (nmol/l)	CV %	Mean (nmol/l)	CV %
2.51	10.2	2.7	11.8
22.9	3.2	23.6	6.4
54.9	3.5	55.8	5.8

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of progesterone.

The mean recovery for added progesterone was 97.3 ± 5.7% in the range 0.79-54.25 nmol/l.

Expected reference values

It is recommended that each laboratory establish its own reference intervals.

As a guide, for follicular phase: 0.6-3.8

nmol/l (0.2-1.2 ng/ml),

luteal phase: 10.5-58 nmol/l (3.3-18.2 ng/ml)

The results obtained should only be interpreted in the context of the overall clinical picture. None of the in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

Additional information

Storage

Store the reagents between 2 and 8°C. At this temperature each reagent is stable until expiry date. Pay special attention to preventing magnetic immunosorbent suspension from freezing.

Availability

From stock.

Shelf life

The shelf life of kit reagents is 8 weeks from the date of manufacturing. To make maximum benefit of long-term stability it is recommended to adjust the date of ordering to labelling calendar issued each year. The actual expiry date is given on package label and in the quality certificate. Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precautions and warnings

This kit should only be used for in vitro diagnostic purposes.

Radioactivity

This kit contains radioactive material. Receipt, acquisition, possession, or use of radioactive materials are subject to regulations, and a licence of (inter)national authorizing bodies. It is the responsibility of the user to ensure that local regulations or codes of practice are satisfied.



Potentially infectious materials



Human blood products provided as components of this product have been obtained from donors tested individually and found negative for Human Immunodeficiency Virus antibody (HIV-Ab) as well as for Hepatitis B surface Antigen (HBsAg) using approved EIA methods.



Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that Hepatitis B Virus, Human Immunodeficiency Virus (HIV), or other infectious agents are absent, and all human blood samples should be considered potentially infectious.



Chemical and other hazard



Some components contain sodium azide (0.1% w/v) as an Antimicrobial Agent. Dispose the waste by flushing it with copious amounts of water to avoid build up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 81 mg.



 Used by  Batch code

 Temperature limitation  Control


 Caution, consult accompanying documents  Standard


 Biological risks  Magnetic Immunosorbent

 Consult instructions for use  Tracer

 *In vitro* diagnostic device  Antiserum

 Manufacturer

 Catalogue number

 Radioactive material

CE

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