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

Description

The PROGESTERONE [I-125] RIA assay system provides the quantitative in vitro determination of Progesterone in human serum. Progesterone can be assayed in the range of 0-120 nmol/L (0-37.7 ng/mL) using 20 µL serum samples. Each kit contains materials sufficient for 100 assay tubes, permitting the construction of one standard curve and assay of 43 unknowns and 1 control in duplicate.

Introduction

Progesterone is one of the C_{21}-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 diagnosis 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 ^{125}I -labelled Progesterone for a limited number of binding sites on Progesterone specific monoclonal 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.

During 1-hour of incubation period with continuous agitation, immuno-complex is immobilized on the reactive surface of test tubes. After incubation the reaction mixture is discarded, tubes are washed and the radioactivity is measured in a gamma counter.

The concentration of antigen is inversely proportional to the radioactivity measured in test tubes. By plotting binding values against a series of calibrators containing known amount of Progesterone, a calibration curve is constructed, from which the unknown concentration of Progesterone in patient samples can determined.

Contents of the kit

1 vial — ^{125}I-TRACER, Ready to use.
11 mL per vial, containing about 260 kBq Progesterone-[^{125}I] in buffer with red dye and 0.1% NaN_3

6 — STANDARDS 1-6, Ready to use.

vials — S_1= 1 mL, S_{2-6}= 0.5 mL per vial, containing 0, 0.8, 3.6, 11, 33, 120 nmol/L in human serum with 0.1% NaN_3

1 vial — ANTISERUM, Ready to use.
11 mL per vial, containing biotinilated monoclonal anti-Progesterone antibody in buffer with blue dye and 0.1% NaN_3.

1 vial — CONTROL SERUM, Ready to use.
0.5 mL human serum with 0.1% NaN_3. The concentration of the control serum is specified in the quality certificate enclosed.

1 bottle — WASH BUFFER CONCENTRATE, 20 mL, with 0.2% NaN_3.
Dilute with 700 mL distilled water before use.

2 boxes — COATED TUBES, ready for use.
2x50 plastic tubes, 12x75 mm, coated with streptavidin.
Quality certificate.
Pack leaflet.

Materials and equipment required

Test tube rack, precision pipettes with disposable tips (20 and 100 µl), shaker, plastic foil, absorbent tissue, gamma counter.
Recommended tools and equipment
Repeating pipettes, dispenser.

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.

Preparation of reagents, storage

Add the wash buffer concentrate (20 mL) to 700 mL distilled water to obtain 720 mL wash solution. Upon dilution store at 2-8°C until expiry date of the KIT.
Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date of the KIT. The actual expiry date is given on the package label and in the quality certificate.

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), zero standard (Standard 1 = B_0), standards (S_{2-6}), control (C) and samples (S_x).
3) Mix all reagents and samples thoroughly before use. Avoid excessive foaming.
4) Pipette 20 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.
5) Seal all tubes with a plastic foil. Fix the test tube rack firmLy onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube (min. 600 rpm).
6) Incubate tubes for 1 hour, shaking at room temperature.
7) Add 2.0 mL of diluted wash buffer to each tube. Decant the solution from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 5 minutes.
8) Count each tube for at least 60 seconds in a gamma counter and calculate the Progesterone concentrations of the samples as described in calculation of results.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microliters). T=total count, S_{1-6} =standards, S_X sample, C=control

Tubes	T	S_{1-6}	C	M_X
Standard 1-6		20		
Control			20	
Samples				20
^{125}-I Tracer	100	100	100	100
Antiserum		100	100	100
Shake for 1 hour at room temperature.				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

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 B_0/T for zero standard (S_1) by using the following equation:

$B_0/T \ \% = 100*S_1/ T$

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

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

Using semi-logarithmic graph paper plot $B/B_0\%$ 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.
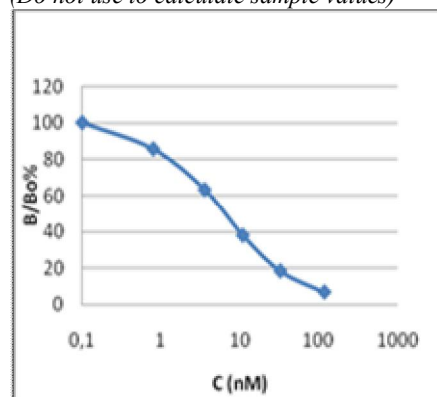
Table 2. Typical Assay Data

Tubes	Mean CPM	B/T %	B/Bo %
T	68202		
S1	53434	78.3	100
S2	45958	67.4	86.0

S3	35030	51.4	65.6
S4	21448	31.4	40.1
S5	10604	15.5	19.8
S6	4051	5.9	7.6
C	11390	16.7	21.3

Figure 1.
A typical standard curve
(Do not use to calculate sample values)

Conversion of SI units

1 nmol/L = 0.3145 ng/mL
1 ng/mL = 3.18 nmol/L

Characterization of the assay

Assay parameters

B_0/T	76 ± 6 %
ED-50	6.7 ± 2 nmol/L

Specificity

Different hormones were added to the "0" standard at two concentration levels (A=70 nmol/L, B=700 nmol/L). The apparent Progesterone concentrations measured are reported below. Nonmeasurable concentrations were obtained for the following hormones added at 700 nmol/L: 17ß-estradiol, 5α dihidro-testosterone, 5ß dihidro-19-nortestosterone, androstendiol, aldosterone, estrone. The DHEA-S cross-reactivity was evaluated at 30 µmol/L, the apparent Progesterone concentration measured was 0.28 nmol/L.

Added steroid concentration	70 nM	700 nM
	Measured Progesterone nM	
17 α metil testosterone	0.25	0.22
Cortisol	0.18	0.25
19-nortestosterone	0.25	0.28
Cortisone	0.21	0.28
Androstandiol	0.21	0.3
Androstendione	0.12	0.34
Deoxicortisol	0.12	0.39
Testosterone	0.1	0.42
17 α OH Progesterone	0.3	0.81
Pregnenolone sulphate	0.35	3.57

Pregnenolone	0.5	4.99
Corticosterone	1.07	12.01
Deoxicorticosterone	1.66	14.21

Sensitivity

Analytical sensitivity is 0.11 nmol/L, as calculated by the interpolation of the mean plus two standard deviations of 20 replicates of the zero standard.

Precision, reproducibility

Six serum samples were assayed in 20 replicates to determine intra-assay precision. To determine inter-assay precision they were measured in duplicates in 20 independent assays. Values obtained are shown below.

Intra-assay		Inter-assay	
mean (nmol/L)	CV %	mean (nmol/L)	CV %
1.87	6.7	1.78	17.3
5.61	3.6	5.33	6.1
17.65	2.4	18.4	3.4
26.35	2.4	27.67	3.1
64.6	2.8	66.6	8.2
80.48	2.2	76.5	5.1

Dilution test (linearity)

Five samples were 2-fold and 4-fold diluted with zero-standard and measured according to the kit protocol. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$$y = 1.0009x - 0.1259 \qquad R^2 = 0.9987 \quad n = 10$$

Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking five serum samples with three different amounts of Progesterone. The mean recovery for added Progesterone was 90.4% ± 4.7% volt (mean ± SD).

Expected reference values

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

Women	N	Minimum (nmol/L)	Maximum (nmol/L)
Follicular phase	31	0.33	5.98
Ovulation peak	21	0.24	4.76
Luteal phase	22	12.9	70.0
Menopausa	49	0.12	2.27
Men	43	0.62	3.75
Children (3-10 y)	64	0.37	3.59

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

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Source of error!** Do not use a shaker where some tubes can be exposed to heating. Do not place the shaker directly by an air conditioning or heating device or by an open window. Any differences in temperature between tubes during incubation can lead to serious measuring errors.

Precautions and warnings

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 used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). 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 infectious agents are absent. Human blood samples should therefore be handled as potentially infectious materials.

Chemical and other hazard

Some components contain sodium azide 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 66 mg.

⌛	Used by	LOT	Batch code
🌡	Temperature limitation	CONTROL	Control
⚠	Caution, consult accompanying documents	CAL	Standard
☣	Biological risks	CT	Coated Tube

	Consult instructions for use	**TRAC**	Tracer
IVD	*In vitro* diagnostic device	**AS**	Antiserum
	Manufacturer	**WASHB**	Wash buffer
REF	Catalogue number		Radioactive material

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