PROGESTERONE$^{[125]I}$ RIA KIT

(Ref: RK-460M)

**Description**

The PROGESTERONE $^{[125]I}$ 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 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 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 $^{125}$I-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-$^{[125]I}$ TME in buffer with 0.1% NaN$_3$

6 vials

STANDARDS, 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$_3$

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.

**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 = B$_0$), standards (S$_2$-S$_6$), 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
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. Keeping the separator inverted, place the tubes on a pad of absorbent tissue and allow to drain for 2 minutes.

12) Count the radioactivity of all tubes preferentially not less than 60 seconds.

13) Calculate the concentrations as described under **Calculation of results**.

### Table 1: Assay Protocol, Pipetting Guide (all volumes in microliters)

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Reagent</th>
<th>T</th>
<th>NSB</th>
<th>S&lt;sub&gt;4&lt;/sub&gt;</th>
<th>S&lt;sub&gt;2&lt;/sub&gt;</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tracer</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Anti-serum</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Vortex mix. Incubate for 2 hours at room temperature.

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Magnetic immunosorbent</th>
<th>500</th>
<th>500</th>
<th>500</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortex mix. Incubate for 15 minutes at room temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Place the tubes on the magnetic separator for 5 minutes or centrifuge for 15 minutes at 1500xg

### Table 2: Typical Assay Data

<table>
<thead>
<tr>
<th>Tubes</th>
<th>Counts CPM1</th>
<th>Counts CPM2</th>
<th>AVG CPM</th>
<th>B/T %</th>
<th>B/Bo %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>45230</td>
<td>46214</td>
<td>45722</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>18517</td>
<td>18728</td>
<td>18623</td>
<td>38.7</td>
<td>100.0</td>
</tr>
<tr>
<td>S2</td>
<td>16281</td>
<td>16068</td>
<td>16175</td>
<td>33.4</td>
<td>86.2</td>
</tr>
<tr>
<td>S3</td>
<td>13515</td>
<td>13284</td>
<td>13400</td>
<td>27.3</td>
<td>70.5</td>
</tr>
<tr>
<td>S4</td>
<td>9294</td>
<td>8980</td>
<td>9137</td>
<td>18.0</td>
<td>46.4</td>
</tr>
<tr>
<td>S5</td>
<td>5003</td>
<td>4844</td>
<td>4924</td>
<td>8.8</td>
<td>22.6</td>
</tr>
<tr>
<td>S6</td>
<td>2694</td>
<td>2880</td>
<td>2787</td>
<td>4.1</td>
<td>10.6</td>
</tr>
<tr>
<td>C</td>
<td>5858</td>
<td>5790</td>
<td>5824</td>
<td>10.7</td>
<td>27.7</td>
</tr>
<tr>
<td>NSB</td>
<td>869</td>
<td>962</td>
<td>915.5</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

### Figure 1

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

<table>
<thead>
<tr>
<th>Progesterone concentration (nM)</th>
<th>B/Bo %</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td></td>
</tr>
</tbody>
</table>

### Conversion of SI units

1 nmol/l = 0.3145 ng/ml
1 ng/ml = 3.18 nmol/l

### Characterization of the assay

**Assay parameters**

<table>
<thead>
<tr>
<th>NSB/T</th>
<th>&lt; 3 %</th>
</tr>
</thead>
</table>

**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/Bo for zero standard (S<sub>1</sub>) by using the following equation:

\[ \text{B/Bo} = \frac{100 \times (S_1 - \text{NSB})}{T} \]

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

\[ \frac{\text{B/Bo} - \text{C}}{\text{S} - \text{C}} \]

### Sensitivity

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

### Precision, reproducibility

<table>
<thead>
<tr>
<th>Intra-assay (1 assay in 9 rep.)</th>
<th>Inter-assay (9 assays in 2 rep.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (nmol/l)</td>
<td>CV %</td>
</tr>
<tr>
<td>2.51</td>
<td>10.2</td>
</tr>
<tr>
<td>22.9</td>
<td>3.2</td>
</tr>
<tr>
<td>54.9</td>
<td>3.5</td>
</tr>
</tbody>
</table>

### 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 nmol/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 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 labeling 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.

WEB site: http://www.izotop.hu
Technical e-mail: immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

INSTITUTE OF ISOTOPES Ltd.
1535 Budapest. Pf.: 851.
Tel.: (+36) 1-392-2577
Fax: (+36) 1-395-9247