Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

\[
\frac{k_a}{k_a} \frac{Ag + AgAbBtn}{Ag + AgAbBtn} \rightarrow \frac{AgAbBtn}{AgAbBtn}
\]

\(k_a\) = Rate Constant of Association

\(k_a\) = Rate Constant of Dissociation

\(K = \frac{k_a}{k_a} = \text{Equilibrium Constant}\)

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effect the separation of the antibody bound fraction after decarilation.

\(AgAbBtn + EnzAgAbBtn \rightarrow \text{immobilized complex}\)

\(\text{Streptavidin} \rightarrow \text{streptavidin immobilized on well}\)

\(\text{Immobilized complex} = \text{sandwich complex bound to the solid surface}\)

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. Utilizing several different serum references of known antigen concentrations, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. u-Estradiol Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for unconjugated estradiol at concentrations of 0 (A), 0.4 (B), 2.0 (C), 5.0 (D), 15 (E), and 30.0 (F) ng/ml. Store at -2 to -8 °C. A preservative has been added. The calibrators can be expressed in molar concentrations (nmol/L) using the conversion factor 3.45. For example: 1ng/ml × 3.45 = 3.45 nmol/L.

B. U-Estradiol Tracer Reagent – 6.0 ml vial
One (1) vial of Estradiol (Analog)-horseradish peroxidase (HPR) conjugate in a protein stabilizing matrix with red dye. Store at 2-8 °C.

C. U-Estradiol Biotin Reagent – 6.0 ml - Icon Y
One (1) bottle of reagent contains anti-conjugated estradiol biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8 °C.

D. Wash Concentrate
One (1) vial contains luminol in a buffer. Store at 2-8 °C.

E. Wash Solution – 20ml - Icon ☑
One (1) vial contains sufficient in buffered saline. A preservative has been added. Store at 2-30 °C.

F. Signal Reagent A – 7.0ml/vial - Icon C
One (1) vial contains luminol in a buffer. Store at 2-8 °C.

G. Signal Reagent B – 7.0ml/vial - Icon D
One (1) vial contains hydrogen peroxide (H2O2) in buffer. Store at 2-8 °C.

H. Product Insert

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8 °C. Kit and component stability are identified on the label.

Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:

1. Pipette capable of delivering 25 µl and 50 µl with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Luminometer.
6. Absorbent Paper for cleaning the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are not absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biological Laboratories," 2nd Edition, 1989, HHS Publication No. (CDC) 89-8395.

Safe disposal of kit components must be according to local regulatory and statutory requirements.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or heparanised plasma in tubes. Samples are taken with use in the collection of venipuncture samples. The blood should be collected in a red-top (with or without gel additives) venipuncture tube or for plasma use evacuated tubes (containing heparin). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8 °C for a maximum period of five (5) days. If the samples cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repeated freezing of the specimen, especially in assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal, and high range for monitoring assay performance. These controls should be treated as unknowns and values determined from every test run should be compared. Quality charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to determine if individual laboratory controls set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviations from established performance can indicate uncontrolled change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Wash Buffer
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer 2-30 °C up to 60 days.

2. Working Signal Reagent A Solution - Store at 2-30 °C
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 ml of A and 1 ml of B per 2 (two) eight well strips (A slight excess of solution is made). Discard the unused portion if not used within 4 hours after mixing. If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27 °C).

*Test procedure should be performed by a skilled individual or trained professional*

1. Format the microplates’ wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 °C.
2. Pipette 0.025 ml (25µl) of the appropriate calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the u-Estradiol Tracer Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of u-Estradiol Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 45 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. An automatic plate washer can be used. Follow the manufacturer’s instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Discard the wash and repeat four (4) additional times.
10. Add 10 µl (100µl) of working signal reagent solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time difference between wells.

DO NOT SHAKE THE PLATE AFTER SIGNAL ADDITION

11. Incubate at room temperature for five (5) minutes in the dark.

12. Read the relative light units in each well with a Chemiluminescence microplate reader for 0.5-1.0 seconds. The results should be read within 30 minutes after adding the working Signal Reagent.

Note: Dilute the sample, subjected to concentrations higher than 30ng/ml, by diluting 1:2 and/or 1:5 with unconjugated estriol ‘0’ ng/ml calibrator or male patient sera with a known value for estriol. Multiply the result by the dilution factor of 2 or 5 as required to obtain the concentration of the sample.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of estriol in unknown specimens.

1. Record the RLU’s obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLU’s for each duplicate serum reference versus the corresponding Progesterone concentration in ng/ml on linear graph paper.
3. Draw a best-fit curve through the plotted points.
4. To determine the concentration of estriol for an unknown, locate the average RLU’s for each unknown on the vertical axis of the graph, for the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged). For example, if the average RLU’s (31308) of the unknown intersects the calibration curve at (0.08) estradiol concentration (see figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertain.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. The addition of signal reagent initiates a kinetic reaction; therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.

6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility and spurious results.

7. Use components from the same lot. No intermixing of reagents from different batches.

8. Patient specimens with Unconjugated E3 concentrations above 30 ng/ml may be diluted (1/2, 1/5 or higher) with unconjugated E3 ‘0’ calibrator and reassayed. The sample’s concentration is obtained by multiplying the result by the dilution factor.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.


13. It is important that the time of reaction in each well is held constant to achieve reproducible results.

14. The % cross reactivity of the Estriol antibody to selected substances, for determination of Unconjugated Estriol, was evaluated by adding the interfering substance to a serum matrix at maximum concentrations. The cross-reactivity was calculated by dividing the dose of interfering substance to dose of Unconjugated Estriol needed to displace the same amount of labeled analog.

TABLE 1

Expected Values for the Unconjugated Estriol CLIA Test System (ng/ml)

<table>
<thead>
<tr>
<th>Estriol Value in ng/ml</th>
<th>Male &amp; Non-Pregnant Female</th>
<th>&lt; 1.0 ng/ml</th>
</tr>
</thead>
</table>

During pregnancy the Unconjugated E3 serum levels rise rapidly, depending on the condition of third trimester (See Table 2 from published Literature).*

TABLE 2

Gestation Week | Expected Range (ng/ml) | Gestation Week | Expected Range (ng/ml) | Gestation Week | Expected Range (ng/ml) | Twin Pregnancy (ng/ml) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>9.5 - 10.2</td>
<td>22</td>
<td>2.7 - 16.0</td>
<td>30</td>
<td>3.0 - 18.0</td>
<td>5.0 - 21.0</td>
</tr>
<tr>
<td>14</td>
<td>0.4 - 1.4</td>
<td>36</td>
<td>2.2 - 21.0</td>
<td>50</td>
<td>5.0 - 25.0</td>
<td>7.0 - 30.0</td>
</tr>
<tr>
<td>16</td>
<td>1.6 - 8.5</td>
<td>40</td>
<td>7.2 - 20.0</td>
<td>100</td>
<td>9.0 - 30.0</td>
<td>13.0 - 40.0</td>
</tr>
</tbody>
</table>

10.8 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be > 1.3.

2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.

2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.

3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.

4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

5. The addition of signal reagent initiates a kinetic reaction; therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.

6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor reproducibility and spurious results.

7. Use components from the same lot. No intermixing of reagents from different batches.

8. Patient specimens with Unconjugated E3 concentrations above 30 ng/ml may be diluted (1/2, 1/5 or higher) with unconjugated E3 ‘0’ calibrator and reassayed. The sample’s concentration is obtained by multiplying the result by the dilution factor.

9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind’s IFU may yield inaccurate results.

10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.


* The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay. In addition, the RLUs of the calibrators have been normalized to 100,000 RU’s for the A calibrator (greatest light output). This conversion minimizes differences caused by variability of the various instruments that can be used to measure light output.

Figure 1

RLUs (A)  
Patient  
Well  
RLUs (B)  
Sample

Table 3

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>20</td>
<td>1.71</td>
<td>4.75</td>
</tr>
<tr>
<td>Sample</td>
<td>20</td>
<td>5.79</td>
<td>3.97</td>
</tr>
<tr>
<td>Sample</td>
<td>20</td>
<td>11.37</td>
<td>3.37</td>
</tr>
</tbody>
</table>

14.3 Accuracy

The unconjugated Estriol AccuLite™ Microplate CLIA Test System was compared with a chemiluminescence immunoassay method. The percentage of agreement between these two methods was determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

Table 4

Between Assay Precision (Values in ng/ml)

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>X</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>20</td>
<td>1.68</td>
<td>0.15</td>
</tr>
<tr>
<td>Sample</td>
<td>20</td>
<td>6.68</td>
<td>0.52</td>
</tr>
<tr>
<td>Sample</td>
<td>20</td>
<td>11.93</td>
<td>0.67</td>
</tr>
</tbody>
</table>

*As measured in ten experiments in duplicate over a ten day period.

14.4 Specificity

The % cross reactivity of the Estriol antibody to selected substances, for determination of Unconjugated Estriol, was evaluated by adding the interfering substance to a serum matrix at maximum concentrations. The cross-reactivity was calculated by dividing the dose of interfering substance to dose of Unconjugated Estriol needed to displace the same amount of labeled analog.

15.0 REFERENCES


Revision: 3 Date: 060412 DCO: 0642
Cat #: 5075-300

For Orders and Inquiries, please contact

Monobind Inc.
109 North Pointe Drive
Lake Forest, CA 92630 USA
Tel: 949-851-2965
Fax: 949-851-3539
Email: info@monobind.com
On the Web: www.monobind.com

Please visit our website to learn more about our other interesting products and services.