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) of repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispensers(s) for conjugate.
4. Microplate washer or a separate bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability
6. Absorbent Paper for blotting 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

The last few years have seen the development of screening for fetal Down Syndrome by measurement of multiple markers in maternal blood. The most common used are the markers unconjugated estriol (u-E3) and PAPP-a (pregnancy associated plasma protein-A).

4.0 REAGENTS
A. u-Estriol Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for unconjugated estriol at concentrations of 0 (A), 0.4 (B), 2.0 (C), 5.0 (D), 15.0 (E), and 30.0 (F) ng/ml can be obtained from the manufacturer. A preservative has been added. The calibrators can be expressed in molar concentrations (mM) by the conversion factor 3.45. For example: 1ng/ml = 3.45 x 10^-9 M

B. u-Estriol Enzyme Reagent – 6.0 ml/vial
One (1) vial of Estriol (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

C. u-Estriol Biotin Reagent – 6.0 ml - Icon
One (1) bottle of reagent contains anti-unconjugated estriol biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml - Icon
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S
One (1) vial contains hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S
One (1) vial contains ultrapure water (H2O). Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions
Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. Opened reagents are shelf 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.

9.0 TEST PROCEDURE
Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-22°C).

1. Format the microplates’ wells for each calorimeter, control and patient specimen to be assayed in duplicate. Replace any unused wells with buffer into the aluminum bag, seal and store at 2-8°C.

2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Add 0.050 ml (50µl) of the U-Estriol Enzyme Reagent to all wells (see Reagent Preparation Section).

4. Swirl the microplate gently for 20-30 seconds to mix.

5. Add 0.050 ml (50µl) of U-Estriol Biotin Reagent to all wells.

6. Swirl the microplate gently for 20-30 seconds to mix.

7. Cover and incubate for 60 minutes at room temperature.

8. Discard the contents of the microplate by decantation or aspiration. Do not blot the plate dry with absorbent paper.

9. Add 50µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). Always add reagents in the same order to minimize reaction time differences between wells.

11. Incubate at room temperature for fifteen (15) minutes.

12. Add 0.050ml (50µl) of stop solution to each well and gently mix for thirty (30) seconds. Do not add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm) and record. Always read reagents in the same order to minimize reaction time differences between wells.

Note: Dilute the sample, suspected of 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 low 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 unconjugated estriol in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader.

2. Plot the absorbance for each duplicate serum reference and control, and a known standard, on a dose response curve.

3. Connect the points with a best-fit curve. To determine the concentration of unconjugated estriol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph. Find the dose response curve at (4.71 ng/ml) unconjugated estriol concentration (See Figure 1). Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.
11.0 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 substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of components should be used.

12.2 Interpretation

1. Measurements and interpretation of results must be performed by a skilled individual or trained professional.
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled Data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the Unconjugated Estriol AccuBind™ ELISA Test System are detailed in Table 1.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Expected Values for the Unconjugated Estriol EIA Test System (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male &amp; Non-Pregnant Female</td>
<td>≤ 0.9 ng/ml</td>
</tr>
</tbody>
</table>

During pregnancy the Unconjugated E3 serum levels rise rapidly till the end of third trimester. (See Table 2 from published Literature.)

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Estimated Weekly Mean (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation Week</td>
<td>Expected Values (ng/ml)</td>
</tr>
<tr>
<td>12-16</td>
<td>0.3 - 1.0</td>
</tr>
<tr>
<td>16-20</td>
<td>1.4 - 6.5</td>
</tr>
<tr>
<td>20-24</td>
<td>1.6 - 8.5</td>
</tr>
</tbody>
</table>

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Unconjugated Estriol AccuBind™ Microplate EIA Test System were determined by analyses on the 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>
<thead>
<tr>
<th>TABLE 3</th>
<th>Within Assay Precision (Values in ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>X</td>
</tr>
<tr>
<td>Low</td>
<td>24</td>
</tr>
<tr>
<td>Normal</td>
<td>24</td>
</tr>
<tr>
<td>High</td>
<td>24</td>
</tr>
</tbody>
</table>

15.0 REFERENCES


*The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a standard curve prepared with each assay.