ESTRAMET™ Urinary 2MeoE ELISA Kit

Cat. No.:DEIA6240
Pkg.Size:96T

**Intended use**

The ESTRAMET™ Urinary 2-Methoxyestrogen Metabolite kit provides materials for the quantitative measurement of total urinary 2-methoxyestrogens (2MeoE) in the urine of males and females.

**Principle Of The Test**

The present test may be described as a competitive, solid-phase enzyme immunoassays (EIA). In this assay format, the antibody is captured on the solid phase and the antigen (estrogen metabolite) is labeled with the enzyme. In the test, binding of the antigen-enzyme conjugate by the antibody is inhibited by free antigen (in the standards, or test sample). Since a restricted number of antibody binding sites are available, the enzyme activity bound to the solid phase in the presence of free antigen is lowered. When enzyme substrate is added to the washed solid phase, the enzyme product (e.g., colored dye) concentration is inversely proportional to the concentration of the free antigen. In the current assay kits, monoclonal antibody to estrogen metabolites are captured directly to the solid phase (wells of 8 x 12 polystyrene microtiter plates). The estrogen metabolite 2MeoE has been conjugated to alkaline phosphatase enzyme (AP).

The urinary forms of 2MeoE are found mainly as the 3-glucuronide and the 3- or 3,16β-glucuronides, respectively. Glucuronides require removal of the sugars before recognition by the monoclonal antibody in the EIA kit. The estrogens are deconjugated of both glucuronic acid and sulphate by use of a mixture of s-glucuronidase and arylsulphatase enzyme isolated from the snail Helix pomatia (mixture is called here "Deconjugating Enzymes"). In practice, an aliquot of urine is diluted 1:20 with a buffer containing the enzymes and incubated until deconjugation is complete. The enzyme digest is then neutralized and used directly in the assay.

**Reagents And Materials Provided**

CHEMICAL HAZARD: Some of the reagents in this kit contain sodium azide as a preservative. For all such solutions, the concentration of sodium azide is 0.05% or less. Sodium azide may react with metals including lead and copper plumbing to form explosive azide salts. Dispose of reagents by flushing down the drain with large volumes of water.

1. Deconjugating Enzymes (21 mL/vial): Enzyme from Helix pomatia with glucuronidase and sulphatase activities. Store at 4-8°C. Contains 0.05% azide as preservative.
2. Neutralization Buffer (22 mL/vial): A buffered solution to neutralize the acidic deconjugation reaction. Store at 4-8°C. Contains 0.05% azide as preservative.
3. 2MeoE:AP Conjugate Diluent (10 mL/vial): A buffered solution to dilute 2MeoE: alkaline phosphatase conjugates. The Conjugate Diluent solution contains monoclonal antibody to 2MeoE (blue-colored). Store at 4-8°C. Contains 0.05% azide as preservative.
4. AP Substrate (22 mL/vial): A basic solution of stabilized paranitrophenyl phosphate (pNPP). Store at 4-8°C.
5. Microtiter Plate (96-well plate with adhesive covers): One plate coated with antibody to mouse immunoglobulin to capture specific antibody-estrogen: alkaline phosphatase enzyme complex. Store at 4-8°C. Contains 0.05% azide as preservative.
6. 2-MeoE:Alkaline Phosphatase Conjugate (5-10 μL in 500 μL/vial). Store at -20°C.
7. Positive Control Urine (0.3 mL). Store at or below -20°C.
8. Standards (0.3 mL): 10, 5, 2.5, 1.25, 0.625, and 0.3125 ng/ml 2-methoxyestrone in 1mL vial. Store at or below -20°C.
9. Sample Diluent (1 mL): Used to dilute premenopausal urine 1:4 with before assay, or if assayed values are found to be greater than 10 ng/mL. Store at or below -20°C.
10. Microtubes (one box): 1.1 mL racked, 96 tubes/rack (do not substitute).
11. Plate Sealers (3 each): Adhesive.

**Materials Required But Not Supplied**

1. The following equipment is absolutely required for accurate results:
   1.1 An individual pipette able to accurately deliver 10 μL
   1.2 An 8- or 12-channel pipette able to accurately deliver 75–200 μL/channel
   1.3 An automatic microtiter plate washer
   1.4 A pH meter capable of measuring to the tenth’s place
   1.5 An automated plate reader with 405 nm filter capable of accurate endpoint and/or kinetic analyses of data with automatic curve fitting by four-parameter fit or equivalent.
2. Additional materials are required to create a plate washing buffer. The recipe for 1 Liter of the Tris Buffer Saline (TBS), pH 7.4/0.05% Tween-20 buffer (use of pre-titrated Tris is highly recommended) used for all plate washing steps is below. Add components to a 1 L container in the order indicated.
   2.1 1 L of glass distilled or deionized water
   2.2 1.51 grams of Tris (10 mM) - use Trizma, pH 7.4, Sigma P/N T-7693 or equivalent
   2.3 8.76 grams of NaCl (150 mM)
   2.4 Check pH and adjust solution to pH 7.4 with 1 N HCl, if necessary
   2.5 0.5 grams Tween-20 (0.05% weight:volume)

**Storage**

PLEASE NOTE: Upon arrival of kit, please remove standards, sample diluent, positive controls, and alkaline phosphatase conjugate from the kit and store at -20°C until use. Store all other components, including plates, at 4-8°C until use. (DO NOT FREEZE PLATES). Do not reuse standards after thawing. The date of kit expiration is typically 90 days after the kit lot number (month/date/yr) if components are stored properly. Do not mix and match components from different lots of kits.

**Specimen Collection And Handling**

Either 24-hour or first-morning void collections are recommended. Freeze/thaw samples at least once before use in the EIA. Urine for long-term storage is best collected with addition of ascorbic acid to urine to prevent oxidation of labile metabolites. 50 mg of ascorbic acid may be added to 50 mL of urine (1 mg/mL). Addition of greater than 5 mg/mL ascorbate may interfere with determination of creatinine. However, untreated urine stored at 4°C for up to 48 hours after collection may be used for assay after freezing and thawing once. The urine container should be labeled, immediately placed at 4°C, and frozen at or below -20°C within 48 hours. Samples without ascorbate may be stored frozen at -20°C for several months without loss of metabolites. Avoid long-term storage of frozen urine samples in frost-free refrigerators and multiple freeze-thawing before assays. Remove all precipitate by centrifugation before use in the assay.

**Typical Standard Curve**

Below is a typical standard curve for EIA of urinary 2MeoE. We highly recommend use of a four-parameter curve to fit the standards.

Figure: Standard curve for EIA of urinary 2-methoxyestrogen; nanograms/mL versus optical density at 405 nm (O.D.units) at 60 minutes after addition of enzyme substrate (para-nitrophenyl phosphate).
Specificity

<table>
<thead>
<tr>
<th>Estrogen Metabolite</th>
<th>Common Name</th>
<th>% Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,5[10]-Estratiren-3-ol-17-one 2-methyl ether</td>
<td>2-Methoxyestrone</td>
<td>100.0</td>
</tr>
<tr>
<td>1,2,5[10]-Estratiren-3,17-ol 2-methyl ether</td>
<td>2-Methoxyestradiol</td>
<td>100.0</td>
</tr>
<tr>
<td>1,2,5[10]-Estratiren-3,16α,17β-triol 2-methyl ether</td>
<td>2,3-Methoxyestrone</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>1,2,5[10]-Estratiren-2,3-diol-17-one 2,3-dimethyl ether</td>
<td>2-Hydroxyestrone</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>1,2,5[10]-Estratiren-2,3-diol-17-one</td>
<td>Estrone</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>1,2,5[10]-Estratiren-3-ol-17β-diol</td>
<td>Estradiol</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>1,2,5[10]-Estratiren-3,16α-diol-17-one</td>
<td>16α-Hydroxyestrone</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Affinity (Ka, L/mol) = 1 x 10⁻²

REFERENCES